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Role of TL1A in the Pathogenesis of Rheumatoid Arthritis

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TNF-like ligand 1A (TL1A), a member of the TNF superfamily, is the ligand of DR3 and DcR3. Several types of cells, such as endothelial cells, monocytes/macrophages, dendritic cells, and CD4 and CD8 T cells, are capable of producing this cytokine. In present study, we demonstrated that TL1A aggravated collagen-induced arthritis in mice. It increased collagen-induced arthritis penetrance and clinical scores as well as the severity of the pathological findings. TL1A administration led to the occurrence of multiple enlarged germinal centers in the spleen, and it boosted serum anti-collagen Ab titers in vivo. In vitro, TL1A augmented TNF-α production by T cells upon TCR ligation, and it greatly enhanced Th17 differentiation and IL-17 production. We further showed that human rheumatoid arthritis (RA) synovial fluids had elevated TL1A titers, and human chondrocytes and synovial fibroblasts were capable of secreting TL1A upon TNF-α or IL-1β stimulation. Taken together, these data suggest that TL1A secretion in lymphoid organs might contribute to RA initiation by promoting autoantibody production, and TL1A secretion stimulated by inflammatory cytokines in RA joints might be a part of a vicious circle that aggravates RA pathogenesis. The Journal of Immunology, 2009, 183: 5350–5357.

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†Abbreviations used in this paper: RA, rheumatoid arthritis; BTIIC, bovine type II collagen; CIA, collagen-induced arthritis; OA, osteoarthritis; RT-qPCR, reverse transcription-quantitative PCR; SF, synovial fibroblast; TL1A, TNF-like ligand 1A.

Changes in the joints lead to the eventual cartilage and bone damages and loss of mobility at a later stage of the disease (5–7).

TNF-like ligand 1A (TL1A), a member of the TNF superfamily of cytokines, is designated as TNFSF15. It is coded by the TNFSF15 gene located on chromosome 9q32 in humans. TL1A is coded by all four exons of TNFSF15. Its mRNA is 2.02 knt in length, containing an open reading frame of 251 aa. However, the 3.3-kb continuous DNA sequence from the beginning of the third exon until the end of the fourth exon of TNFSF15 encodes a different protein called vascular endothelial growth inhibitor, or TL1 (8). X-ray crystallography shows that TL1A proteins form homotrimers in solution (9), like other TNF family members.

Initially, TL1A was thought to be produced only by endothelial cells, and its production was induced by TNF-α and IL-1β (8). Further studies have shown that it is also secreted by monocytes/macrophages and dendritic cells upon immune complex stimulation, and it is constitutively expressed in NKT cells; it is also present in human synovial tissue and exudates (10–12). In certain chronic inflammatory conditions, such as Crohn’s disease, CD4 and CD8 T cells produce this cytokine (13). A repertoire of all TL1A-producing cells has not yet been completed.

TL1A binds to two proteins, DcR3 and DR3, both of them being TNFR family members. DcR3 is a secreted protein. As such, it does not transduce signals into cells, but simply interferes with interactions between several ligands and receptors, such as FasL/Fas, LIGHT/LTβR, LIGHT/HVEM, and TL1A/DR3 (8, 14–16). The DR3 gene produces several alternative splicing variants, which can be membrane-bound as well as in secreted form (17, 18). DR3 is expressed on T cells and NK cells and is up-regulated upon their activation (17, 19). Its expression is induced by TGFβ1 and, hence, Th17 cells present especially high DR3 levels (20). It is detectable in B cells, osteocytes, and renal tubule epithelial cells (21–23). In DR3-null mutant mice, thymocytes experience compromised negative selection (24). T and B cell populations are normal in the peripheral lymphoid organs of these mice, but detailed studies on the pathogenesis of various diseases have not been conducted.

Soluble TL1A increases IFN-γ and GM-CSF secretion in T cells with TCR ligation, and it synergizes with IL-12 to enhance IFN-γ...
production in T and NK cells (25). TL1A enhances the ability of NKT cells to secrete IL-13. Mice receiving anti-TL1A Ab or transgenic mice with T cells/NK cells expressing dominant-negative TL1A are resistant to lung inflammation (10). TL1A also promotes adhesion of monocytes to endothelial cells, and it presumably enhances their extrudes to extravascular space (26). Intracellular IL-17+ CD4 cells are increased when TL1A is present in a condition favoring Th17 differentiation. Conversely, TL1A-null mutant DC cells have a reduced capacity of supporting Th17 cell differentiation (20). Furthermore, TL1A-null mutant mice are resistant to experimental autoimmune encephalitis induction, which requires Th17 (20). These in vitro and in vivo data have demonstrated that TL1A is an important mediator of inflammation.

In this study, we explored the role of TL1A in RA pathogenesis. TL1A administration exacerbated collagen-induced arthritis (CIA) and augmented serum Abs against collagen in a mouse model. Further mechanistic experiments showed that fibroblast-like synoviocytes were capable of producing TL1A upon TNF-α and IL-1β stimulation, and TL1A, in turn, enhanced the production of inflammatory cytokines, including TNF-α and IL-17, by T cells. The implications of these findings in RA pathogenesis are discussed.

Materials and Methods

Reagents

Recombinant human TL1A (aa 72–251) was produced in Chinese hamster ovary cells and subsequently processed to >95% purity, as described earlier (8). Recombinant mouse IL-1β, TNF-α, TGF-β1, and IL-6 were purchased from R&D Systems. Neutralizing mAbs against IPN-γ and IL-4 were obtained from R&D Systems. PE-conjugated anti-IL-17 mAb was from BD Biosciences.

Mouse CIA model

Eight- to 10-wk-old DBA/1Jc male mice (The Jackson Laboratory) were used for this study. The Human Genome Sciences’ Institutional Animal Care and Use Committee approved all experimental protocols used in this work.

The scheme of the mouse CIA model is depicted in Fig. 1A. Briefly, DBA/1Jc mice were immunized at the base of the tail with 100 μg of bovine type II collagen (BTTIC; Chondrex), which was emulsified in equal volumes of Freund’s complete adjuvant (2 mg/ml Mycobacterium tuberculosis, strain H37Ra; Difco) on day 0. Three groups of immunized mice were then injected i.p. daily with buffer (PBS), TL1A (1 mg/kg/day), or heat-denatured TL1A (HD-TL1A), respectively, from day 5 to 20. As a positive control, a fourth group was challenged on day 15 with a single s.c. injection of LPS (60 μg/mouse). The mice were examined for the development and severity of arthritis from day 5 to 50. Disease severity was scored on a scale from 0 to 3 by visual inspection of paws, and score criteria were as follows: 0.5, one or more swollen digits; 1.0, entire paw swollen; 2.0, deformity following acute edema; and 3.0, ankylosis (i.e., total loss of joint function). The scoring was done by two independent observers without knowledge of the experimental groups. The scores for each of four paws were added together to give a final score, such that the maximal severity score could be 12 for a given mouse. The mice were considered as having arthritis if their clinical score was ≥0.5.

Histology

After sacrificing animals on day 50, limbs (including the paw and ankle) were surgically removed and fixed in 10% buffered formalin. Following decalcification in 5% formic acid, the specimens were processed for paraffin embedding. Tissue sections (7 μm) that were stained with H&E were scored by a certified pathologist for mean inflammation, pannus formation, cartilage damage, and bone damage. The overall score was based on a set of three to four joints per animal and all were scored on a 0–5 scale, as previously described (27). A mean score for each animal was determined for each parameter, and these were averaged to determine group means.

Chondrocyte culture

Human osteoarthritis (OA) cartilage samples from femoral condyles and tibial plateaus were obtained from OA patients undergoing total knee replacement (n = 4; mean ± SD of age, 64 ± 11 years). All OA patients were diagnosed according to the criteria developed by the American Col-lege of Rheumatology Diagnostic Subcommittee for OA (28). At the time of surgery, the patients had symptomatic disease requiring medical treatment in the form of nonsteroidal anti-inflammatory drugs or selective cyclooxygenase-2 inhibitors. Patients who had received intraarticular injections of steroids were excluded. The Clinical Research Ethics Committee of Notre-Dame Hospital approved the study protocol and the use of human articular tissues.

Chondrocytes were released from cartilage by sequential enzymatic digestion, as described previously (29). In brief, this consisted of 2 mg/ml pronase for 1 h followed by 1 mg/ml type IV collagenase (Sigma-Aldrich) for 6 h at 37°C in DMEM and antibiotics (100 U/ml penicillin, 100 μg/ml streptomycin). The digested tissue was briefly centrifuged and the pellet was washed. The isolated chondrocytes were seeded at high density in tissue culture flasks and cultured in DMEM supplemented with 10% heat-inactivated FCS. At confluence, the chondrocytes were detached, seeded at high density, and allowed to grow in DMEM, supplemented as above. The culture medium was changed every second day, and 24 h before the experiment, the cells were incubated in fresh medium containing 0.5% FCS. Only first-passage chondrocytes were used.

Synovial fibroblast (SF) culture

Human SF was isolated from synovial membranes obtained from OA patients undergoing total knee replacement as described above. They were released by sequential enzymatic digestion with 1 mg/ml pronase (Roche Applied Science) for 1 h, followed by 6-h incubation with 2 mg/ml type IV collagenase (Sigma-Aldrich) at 37°C in DMEM supplemented with 10% heat-inactivated FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin. The cells were incubated for 1 h at 37°C in Primaria 3524 tissue culture flasks (BD Falcon), allowing the adherence of nonfibroblastic cells possibly present in the cell preparation. After removing the adherent nonfibroblastic cells, nonadherent cells were seeded in tissue culture flasks and cultured until confluence in DMEM supplemented with 10% FCS and antibiotics. Only cells between passages 3 and 7 were used.

Human synovial fluids

Normal human synovial fluids were obtained at necropsy, within 12 h of death, from four donors (54 ± 8 years) with no history of arthritic diseases. RA synovial fluids were obtained from seven patients (57 ± 14 years, mean ± SD) undergoing total knee replacement. All patients were diagnosed on criteria developed by the American College of Rheumatology. Patients who had received intraarticular injections of steroids were excluded.

ELISA

For TL1A assays, flat-bottom 96-well plates (Costar EL/A/Ria plate no. 3590; Fisher Scientific) were coated with anti-TL1A capture mAb (15E09) (50 μl/well at 3 μg/ml diluted in NaHCO3 buffer (pH 9.0), and incubated overnight at 4°C. After blocking for 1 h at room temperature with 5% BSA in PBS, the plates were washed with washing buffer (0.05% Tween 20 in PBS). Samples (50 μl/well) were added to the wells and incubated overnight at 4°C. After an extensive wash, 50 μl/well affinity-purified, biotinylated anti-TL1A-rabbit polyclonal Ab at 0.1 μg/ml was added and incubated at room temperature for 2 h. Fifty microliters per well of horseradish peroxidase-conjugated streptavidin (R&D Systems) diluted 1/200 in washing buffer was added after extensive washing. One hour later, the plates were washed, and 50 μl/well tetramethylbenzidine (Sigma-Aldrich) was added and incubated for 30 min at room temperature in the dark. Fifty microliters per well of 2 N H2SO4 was added to stop the reaction, and optical densities were determined at 450 nm. Samples were assayed in duplicate.

To detect serum anti-bovine collagen Ab (total IgG and IgG2a), mice were tail bled before being sacrificed on day 50. Serum samples were analyzed with ELISA kits from Chondrex following the manufacturer’s instructions.

IPN-γ, TNF-α, IL-2, and IL-17 were measured by ELISA with kits from R&D Systems, according to the manufacturer’s instructions. Samples were assayed in triplicate.

Reverse transcription-quantitative PCR (RT-qPCR)

Total RNA was isolated from chondrocytes, SF, or mouse joints with TRIZol reagent. TL1A and DR3 mRNA in human chondrocytes, SF, and mouse joints were measured by RT-qPCR. For human TL1A mRNA measurement, the forward primer (5'-AGGACAGGAGTGTGACCCCTCCA-3') and reverse primer (5'-AGTGCTGTGTTGGAGTTTGTCTCA-3') were
employed to generate a 110-bp fragment. For mouse TL1A mRNA measurement, the forward primer (5'-TACATTCTCATCCTCGAGAAGCTT-3') and reverse primer (5'-GGAAGGAGAACAGCAACGAATG-3') were employed to generate a 166-bp fragment. For DR3 human mRNA measurement, the forward primer (5'-ACTCAGGGCGGAAATGTTGA GAAAT-3') and reverse primer (5'-ACTTCCATCATCCTCGGCTCTGCT-3') were employed to generate a 66-bp fragment. The PCR conditions for these reactions were as follows: 2 min at 95°C, followed by 45 cycles of 10 s at 95°C and 15 s at 56°C, and 20 s at 72°C. Samples were in triplicate. β-actin mRNA levels were taken as internal controls, and the data were expressed as signal ratios of TL1A mRNA/β-actin mRNA and DR3 mRNA/β-actin mRNA.

**In vitro Th1, Th2, and Th17 polarization**

Th1 and Th17 populations were polarized from naive DBA/IiLacJ CD4+ T cells, which were isolated from pooled splenocytes and lymph node cells using the Naive CD4+ T Cells Isolation kit (R&D Systems). The purity of naive CD4+ T cells (0.25 × 10^5/well) were mixed with T cell-depleted irradiated (3000 rad) DBA/IiLacJ feeder splenocytes (1.25 × 10^6 cells/well) and cultured in 96-well plates in RPMI 1640 medium containing 10% FCS, 100 U/ml streptomycin, 100 U/ml penicillin G, 1× nonessential amino acids, 1 μM sodium pyruvate, 2.5 μM 2-ME, and soluble anti-CD3 mAb (clone 145-2C11, 2 μg/ml). Th2 polarization was started with total CD4+ T cells, which were purified with an EasySep Mouse CD4 Positive Selection kit (StemCell Technologies). Purified total CD4+ T cells (0.4 × 10^6/well) were mixed with irradiated (3000 rad) T cell-depleted DBA/IiLacJ feeder splenocytes (0.2 × 10^6 cells/well) and cultured in 96-well plates in the same medium used for Th1 and Th17 polarization.

For Th1 polarization, 10 ng/ml recombinant mouse IL-12 and 10 μg/ml anti-IL-4 mAb (clone 11B11) were added to the culture. For Th2 polarization, 20 ng/ml recombinant mouse IL-4 and 10 μg/ml both anti-IL-12 mAb (10 μg/ml; BD Biosciences) and anti-IFN-γ mAb (10 μg/ml; R&D Systems) were added. For Th17 polarization, cultures were supplemented with recombinant mouse IL-6 (20 ng/ml; R&D Systems), recombinant human TGF-β1 (5 ng/ml; R&D Systems), and anti-IL-4 and anti-IFN-γ mAb (10 μg/ml; R&D Systems). Recombinant human TL1A was added at concentrations of 0, 40, 200, and 1000 ng/ml.

For IL-17 assays, cells were washed and stimulated with 5 nM PMA and 500 ng/ml ionomycin in the last 4 h of 3 days of culture. The supernatants were then collected for cytokine analysis. For IL-12 and IL-4 assays, the cells were kept in culture for 5 days and then stimulated with 5 nM PMA, 500 ng/ml ionomycin, and 50 U/ml recombinant human IL-2 in the last 12 h, and the following steps were the same as those of IL-17.

**Results**

**TL1A aggravated CIA in mice**

With mouse CIA as a model, we investigated the roles of TL1A in disease development. DBA/IiLacJ mice were immunized with BTIIC in CFA. A group was injected with PBS as negative controls, which did not develop any disease, as expected. Sixty percent of the BTIIC-immunized mice (CIA-PBS group) developed CIA within 50 days (Fig. 1B). Disease onset followed a slow kinetics, as CIA was not detected before day 24, and at day 40, only 35% of the mice showed CIA. As a positive control, a group of BTIIC-immunized mice was boosted with LPS (CIA-LPS group) on day 15, and it induced a rapid onset of arthritis within 2 days; that is, on day 16, 80% of the mice became arthritic, and on day 20, 90% became arthritic. When human recombinant TL1A (in all experiments of this study, the recombinant TL1A used was of humans)
was administered from day 5 to 20 (CIA-TL1A group), it significantly aggravated CIA induction. The disease started its manifestation earlier (day 20), compared with BTIIC immunization alone (day 24), and presented 100% penetrance on day 40, compared with only 35% for BTIIC immunization alone. On the other hand, BTIIC-immunized mice treated with heat-inactivated TL1A (CIA-HDTL1A group) showed disease development kinetics similar to those of BTIIC immunization alone. This ruled out the possibility that the aggravated CIA in TL1A-injected mice (CIA-TL1A group) was due to pyrogen contamination in the TL1A preparation, or due to its nonspecific effect as a foreign Ag, as the recombinant human TL1A was used in our experiment.

The data are also presented in the form of clinical scores (Fig. 1, C and D). Throughout the 50-day observation period, the BTIIC-immunized group did not present a mean clinical score above 2. Administration of heat-denatured TL1A from day 5 to 20 had no obvious effect (Fig. 1C). In the positive control group with LPS injection at day 15, the clinical score rapidly increased from 0 to a mean of 2.5 in 2 days. In the group that received TL1A for 15 days from day 5 to 20, the clinical score was significantly elevated, as its mean scores reached 3.0 on day 38 and 5.0 on day 50, compared with the CIA-PBS control group (Fig. 1C). At the end of the study on day 50, the score of this test group was significantly higher than that of the CIA-PBS group and the CIA-HDTL1A group (p = 0.0006 and p = 0.004, respectively) (Fig. 1D).

The histopathology of each treatment group was scored semiquantitatively, and the results are depicted in Fig. 2B. While CIA-PBS and CIA-HDTL1A groups showed no difference in their scores, the score of the CIA-TL1A group was significantly higher than those of these two groups.

FIGURE 2. Effect of TL1A on CIA histology. A, Histology of mouse joints. DBA/1LacJ mice were immunized with BTIIC to induce CIA in the absence or presence of TL1A injection. They were sacrificed on day 50 and the hind paws were sectioned and stained with H&E. Left panel, Hind paw from a CIA-PBS mouse; right panel, hind paw from a CIA-TL1A mouse. B, Histopathological scores of mouse joints. Mice from Fig. 1B were sacrificed on day 50, and joint histology was scored semiquantitatively. The horizontal bars represent the mean scores of each group (n = 10). The histopathological scores of the CIA-TL1A group are significantly higher than those of the CIA-PBS and CIA-HDTL1A groups (p = 0.0006 and p = 0.002, respectively; ANOVA t test).

FIGURE 3. TL1A enhances humoral immune responses. A and B, Effect of TL1A on serum collagen-specific Abs. Sera from mice as described in Fig. 1B were collected at the time of sacrifice (day 50 after initial BTIIC immunization). Collagen-specific IgG Ab (A) and IgG2a Ab (B) were measured by ELISA. The data are expressed as U/ml of 1/100 diluted sera. The horizontal bars represent the means of each group (n = 20). Serum collagen-specific IgG and IgG2a levels in the CIA-TL1A group are significantly higher than those in the CIA-PBS and CIA-HDTL1A groups (p values are indicated in the graphs; ANOVA t test). C and D, TL1A drastically increases spleen germinal center formation. DBA/1LacJ mice were injected i.p. with TL1A (3 mg/kg/day) for 10 days, and spleen histology is shown. C, Normal spleen from a naive mouse. D, Spleen from a TL1A-injected mouse.
Note that LPS-treated mice presented high clinical but low pathological scores. LPS triggers innate immune responses and inflammatory cytokine releases. It mainly causes swelling and loss of function of joints, but results in little cartilage and bone damage. Thus, LPS-treated mice showed high clinical scores, which were mainly based on joint swelling and function. On the other hand, severe pathological scores were based on pannus formation, cartilage damage, and bone damage, which were absent in LPS-treated mice.

**TL1A increased humoral immune responses in vivo**

Collagen-specific autoantibodies are pathogenic in RA and are directly disease-causative in CIA. Serum collagen-specific IgG and IgG2a levels were measured at the end of the study on day 50 in mice receiving different treatments (Fig. 3). Naive mice (naive-PBS group) presented no anti-collagen IgG (Fig. 3A) and IgG2a Abs (Fig. 3B). The CIA-PBS and CIA-HDTL1A groups had similar levels of collagen-specific IgG and IgG2a Abs, but these Abs were elevated in the CIA-LPS group, as expected. In the CIA-TL1A group, levels of these Abs were significantly higher than those of the CIA-PBS group (for both IgG and IgG2a, *p* < 0.05) and the CIA-HDTL1A group (*p* < 0.01).

Secondary lymphoid organs, such as the spleen, are the place where B cells undergo activation and differentiation and become Ab-producing plasma cells. We examined the effect of TL1A on spleen histology. As shown in Fig. 3C, in the normal spleen (left panel), small germinal centers were occasionally present in the white pulp. However, in the spleen of mice treated with TL1A (3 mg/kg/day for 10 days without any additional immunization), there were many enlarged germinal centers displaying lightly stained areas, which might have been due to cell proliferation. This suggests that TL1A could promote humoral immune responses, and it explains the significantly augmented anti-collagen Ab levels in mice with BTIIC immunization followed by TL1A treatment.

**TL1A expression in synovial fluids and cells in joints**

Inflammatory cytokines locally produced in joints play an important role in RA pathogenesis. To test whether TL1A was produced locally in joints, we first assessed its presence in RA synovial fluids. As shown in Fig. 4A, TL1A was below the detection level
in synovial fluids of four non-RA individuals. On the other hand, six out of seven synovial samples from RA patients showed elevated TL1A levels.

We also tested TL1A expression in joints from CIA mice and found that its mRNA level was significantly augmented, compared with that of joints of naive mice (Fig. 4B), suggesting the involvement of local TL1A in CIA pathogenesis.

To identify the cell source of synovial fluid TL1A, human chondrocytes and SF were cultured in plain medium or in the presence of inflammatory cytokines IL-1β or TNF-α, and their TL1A mRNA levels were quantified by RT-qPCR. As shown in Fig. 4C, chondrocytes in the resting state had barely detectable TL1A mRNA, but its expression increased in the presence of TNF-α but not IL-1β after 24 h. SF presented higher constitutive TL1A mRNA level, and the expression increased slightly after IL-1β treatment, but significantly after TNF-α stimulation (Fig. 4C). Careful kinetic study revealed that TL1A mRNA was rapidly elevated in SF within 3–6 h after IL-1β stimulation, while it took 20 h to reach high levels after TNF-α stimulation (Fig. 4D). At the protein level, both IL-1β and TNF-α dose-dependently enhanced TL1A secretion by SF at 24 h after culture (Fig. 4E, top panel), and 48-h supernatants contained more TL1A than 24-h supernatants (Fig. 4E, bottom panel). This result revealed that SF was a source of local TL1A, which might influence inflammation in situ in the joints.

On the other hand, neither chondrocytes nor SF showed apparent constitutive DR3 mRNA expression, nor did they up-regulate DR3 expression after IL-1β and TNF-α stimulation (Fig. 4C), indicating that TL1A was unlikely to exert an autocrine effect on these cells. Indeed, TL1A did not affect proliferation of chondrocytes and SF, nor did it impact their apoptosis (data not shown). Therefore, chondrocytes and SF seemed not to be the intended targets of locally produced TL1A by SF.

**TL1A expression in secondary lymphoid organs**

In mouse CIA, systemic TL1A administration increased titers of anti-collagen Ab, which are pathogenic. In RA patients, particularly in rheumatoid factor-positive patients, their serum TL1A levels are increased (30). This implies that extra-articular TL1A might also be involved in pathogenesis of RA and mouse CIA. We assessed whether TL1A was produced in secondary lymphoid organs in our CIA model. We showed that TL1A expression was induced in MHC class II⁺/CD11c⁺ dendritic cells (Fig. 5A) and F4/80⁺ macrophages/macrophages (Fig. 5B) in draining inguinal lymph nodes 2 wk after tail base injection of CFA or of CFA plus collagen. CFA alone was sufficient to up-regulate TL1A, and the presence of collagen along with CFA further increased TL1A expression in these cells.

**Effect of TL1A on lymphokine production**

In RA, considerable T lymphocyte infiltration occurs in joints once the disease starts. What is the effect of local TL1A on these cells, which are known to express TL1A receptors (i.e., DR3)? We assessed the influence of TL1A on lymphokine secretion by T cells. With TCR ligation, TL1A enhanced TNF-α production in a dose-dependent manner (Fig. 6A). Under a Th17-differentiation condition, the presence of TL1A in culture drastically increased IL-17 secretion (Fig. 6B). It is conceivable that these local inflammatory cytokines, that is, TNF-α and IL-17, triggered by TL1A could aggravate RA pathogenesis. On the other hand, under Th1 and Th2 differentiation condition, the production of Th1 cytokine IFN-γ (Fig. 6C) and Th2 cytokine IL-4 (Fig. 6D) by T cells was not apparently influenced by the presence of TL1A.

**Discussion**

In this study, we reported that TL1A aggravated RA in a mouse CIA model. Mechanistic investigation discerned that under stimulation of inflammatory cytokines, such as IL-1β and TNF-α, SF was capable of producing TL1A, which, in turn, could enhance the production of inflammatory cytokines, such as IL-17 and TNF-α. This study revealed a possible role of TL1A in RA pathogenesis. In our CIA mouse model, the systemic administration of TL1A could have pathogenic effects on two fronts. One front is in the

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**FIGURE 5.** TL1A was up-regulated in dendritic cells and macrophages/macrophages of inguinal lymph nodes of immunized mice. A, TL1A was up-regulated in MHC-II and CD11c double-positive dendritic cells. DBA/1J mice were immunized with CFA plus PBS or CFA plus BTIC and sacrificed on day 14. TL1A expression on inguinal lymph node dendritic cells was assessed by MHC-II, CD11c, and TL1A three-color staining followed by flow cytometry. The MHC-II and CD11c double-positive dendritic cells were gated for TL1A expression analysis. B, TL1A was up-regulated in F4/80⁺ macrophages/macrophages. Cells isolated from inguinal lymph nodes of immunized mice were also stained with Abs against F4/80 and TL1A; expression of TL1A is shown in histogram.
joints locally. As demonstrated in our in vitro study, TL1A promoted T cells to secrete locally TNF-α, an established culprit in RA development (3). It also promoted local IL-17 production, which has also been implicated in RA pathogenesis (4). The second front is probably in the lymphoid organs, such as the spleen, lymph node, and bone marrow, where Abs are produced. TL1A drastically increased the size and number of germinal centers in the spleen, suggesting that it could drive B cell activation and differentiation. Indeed, mice receiving TL1A showed heightened titers of serum anti-collagen Ab, which is pathogenic in both mouse CIA and RA patients (31). The effect of TL1A on B cells is probably indirect via T cells, because B cells display almost no DR3 (8), and we could not detect any direct effect of TL1A on B cell activation and proliferation (data not shown).

Is TL1A a relevant factor involved in RA pathogenesis? In mouse CIA, we identified increased TL1A mRNA in inflammatory joints. In humans, we discovered elevated TL1A titers in RA synovial fluids, and found that SF under the influence of IL-1β and TNF-α could evoke TL1A secretion. This puts TL1A into a possible loop of a vicious circle that aggravates RA pathogenesis. Once RA is in progress, local IL-1β and TNF-α levels are increased in the joints, produced mainly by monocytes/macrophages (32). In addition to their harmful effects in causing local inflammation (33, 34), these cytokines could also trigger SF to produce TL1A, which could, in turn, augment TNF-α production by infiltrating T cells in the joints. The IL-17 induced by TL1A could be another detrimental factor ramified from this vicious loop, causing further local inflammation in the joints.

In a study that was published while our manuscript was under revision, Bamias et al. demonstrated that rheumatic factor-positive RA patients showed elevated serum TL1A titers (30). The increased serum TL1A titers in RA patients suggest that TL1A outside the joints may also play a role in RA pathogenesis. We have shown that bacterial Ag stimulation in the form of CFA was sufficient to trigger TL1A expression in dendritic cells and macrophages in draining lymph nodes. It is possible that under a pathophysiological condition, certain RA-susceptible individuals have increased TL1A titers in secondary lymphoid organs upon environmental Ag stimulation; the augmented TL1A levels enhance the production of Ab, some of which might be autoreactive to joint tissues; this process then initiates RA pathogenesis.

In this regard, it is noteworthy that DR3 gene duplication in chromosome region 1p36.3 is prevalent in RA patients (35), raising the possibility that TL1A and DR3 interaction indeed plays a role in human RA pathogenesis.

In summary, we demonstrated that TL1A is a novel mediator involved in RA pathogenesis and is a possible therapeutic target for this disease.

Note added in proof. While our manuscript was under review, Bull et al. (36) reported that DR3 (the receptor of TL1A) gene
knockout mice were resistant to Ag-induced arthritis. They further demonstrated that anti-TL1A mAb could reduce severity of both Ag-induced arthritis and CIA in mice. These new findings support our conclusion that TL1A might have pathogenic roles in mouse CIA and human RA.

Disclosures

The authors have no financial conflicts of interest.

References