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The adaptor protein CIKS/Act1 is necessary to induce collagen-induced arthritis pathology and it contributes to collagen-specific antibody production

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Abstract

Objective—CIKS/Act1 is an adaptor molecule necessary for signaling by members of the IL-17 cytokine family. Here we aim to determine whether this adaptor is required for collagen-induced arthritis (CIA). If required, CIKS-mediated signaling could be a potential target for therapeutic intervention in rheumatoid arthritis.

Methods—CIA model studies were performed with CIKS deficient and sufficient mice on an otherwise wild-type C57BL/6 background or on a background lacking FcγRIIb. In addition, wild-type and CIKS deficient mice were subjected to collagen-antibody induced arthritis (CAIA) studies. Arthritis pathology was determined by visual inspection of the paws, by histochemical analysis of tissue sections and by measurements of collagen-specific antibodies.

Results—Arthritis pathology could be readily induced with the CIA model in wild-type mice and pathology was exacerbated in FcγRIIb-deficient mice. In contrast, CIKS deficient mice were protected from all aspects of CIA pathology, even in FcγRIIb deficient mice. The absence of CIKS completely prevented neutrophil infiltration into joints, bone erosion and cartilage damage; furthermore, production of collagen type 2-specific antibodies (CII-Abs) was reduced. In contrast to the CIA model, CIKS deficient mice remained susceptible to arthritis induced with the CAIA model.

Conclusion—CIKS-mediated signaling is necessary for the pathogenesis in the CIA model, but not in the CAIA model. These findings suggest critical functions of CIKS during the development of arthritis in the CIA model, including in the formation of CII-Abs, and they mark the CIKS adaptor as a potential therapeutic target in RA.

Keywords

Collagen-Induced Arthritis; Interleukin 17; Signal Transduction; Antibody Production

T helper cells type 17 (Th17) are thought to be critically involved in development of rheumatoid arthritis (RA) as well as in collagen-induced arthritis (CIA), a mouse model of

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Siebenlist had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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RA. Arthritis inducing functions of Th17 cells are mediated in part via production of IL-17A (a.k.a. IL-17), the signature cytokine of Th17 (1,2). IL-17A belongs to a family of six cytokines (IL-17A-F) that signal via receptors composed of members of a family of 5 polypeptides (IL-17RA-RE), although the nature of these receptors is still poorly understood (3-6). IL-17A and IL-17F are closely related and produced by Th17 cells, but both are also secreted by other cells, including $\gamma\delta$ T cells, iNK T cells, and lymphoid tissue inducers, among others (4-9). IL-17B, IL-17C and IL-17D appear to be generated mainly by non-hematopoietic cells (3,4), while IL-17E (more commonly named IL-25) has been reported to be produced by a variety of cell types, including, among others, Th2 cells, mast cells, eosinophils and lung epithelial cells (3,4,10). IL-17A and IL-17F may signal largely via a heteromeric receptor formed by IL-17RA and IL-17RC chains (4,8), while IL-25 (IL-17E) may signal via a heteromeric receptor formed by IL-17RA and IL-17RB, with IL-17RB providing the primary binding surface for IL-25 (4,11,12). Nevertheless, the precise nature of the signaling receptors for IL-17A/F and IL-25 remains to be determined and very little is known about the receptors for the remaining members of this cytokine family.

CIKS (Connection to IKK and SAPK/JNK; a.k.a. Act1) (13,14) is an adaptor protein required for signaling by IL-17A (15-17). CIKS and all members of the IL-17 receptor family contain so-called SEFIR domains (similar expression to fibroblast growth factor [SEF]/IL-17 receptor domain); SEFIR domains are distantly related to TIR domains present on Toll and IL-1 receptors and their adaptors, such as MyD88. Upon signaling by IL-17A CIKS is recruited to the IL-17 receptor complex via heterotypic SEFIR domain-mediated interactions (16,18) which initiates a cascade of events culminating in activation of downstream effectors regulating gene expression, including MAP kinases, NF- κ B and c/EBPs (4,13,14). Recent evidence indicates that CIKS is also required for IL-25 signaling (17,19) and while relatively little is known about the remaining members of the IL-17 cytokine and receptor families, it is reasonable to assume that CIKS is similarly important for their signaling as well.

Much evidence implicates IL-17A as an important mediator of pathogenesis in the CIA model in mice as well as in rheumatoid arthritis patients (2). IL-17A has been identified in RA synovial biopsies (20). Furthermore, mice lacking IL-17A are partially resistant to CIA (21), and are impaired in the development of spontaneous arthritis on an IL-1Ra-deficient background (22). Blocking IL-17A with neutralizing antibodies reduces the severity of CIA (23) and improves signs and symptoms of RA (24). In addition, IL-17F may contribute to arthritis incidence, albeit modestly (25). IL-17B and IL-17C have been reported to contribute to TNF α production and to exacerbate pathology in the CIA model (26).

Since all four of these cytokines are likely to signal via CIKS, this adaptor and the pathways it activates may provide a particularly useful target to combat RA. On the other hand, interfering with IL-17 cytokine signaling could critically impair host defense and render animals susceptible to infections. Furthermore, interference with CIKS function could also impair signaling by IL-25, a Th2 response-associated cytokine that can function to delimit the generation of Th17 cells (17,27). Impaired IL-25 signaling could conceivably enhance Th17-associated functions not related to IL-17A/F, thus possibly exacerbating arthritis. Aside from its role in IL-17 cytokine family signaling, Act1/CIKS has also been proposed to negatively regulate CD40- and BAFF-mediated B cell functions and survival. Loss of Act1 was reported to trigger B cell hyper-reactivity, leading to autoantibody production and even outright autoimmune disease as mice age (15,28). Therefore, loss of Act1/CIKS might exacerbate autoantibody production and thus pathology in CIA. However, the aforementioned blatant B cell hyper-reactivity and resulting autoimmune conditions were not apparent in an independently generated CIKS-deficient mouse model, possibly due to subtle strain and/or environmental differences, as discussed previously (17). Given these

possible scenarios the physiologic role of CIKS in the pathogenesis of CIA remains on open question. The present study seeks to address the relevance of CIKS in the CIA model, especially as this will also help determine whether CIKS-mediated signaling could be a possible target for therapeutic intervention in RA.

We have applied the CIA model to CIKS deficient and CIKS sufficient mice that had either an otherwise wild-type background or were also lacking Fc γ RIIb, with the latter mice displaying exacerbated arthritis pathology (29). We show that CIKS was absolutely necessary for the development of pathology in the CIA model on both genetic backgrounds. In contrast to CIA, CIKS had no role in the acute arthritis condition induced by administration of anti-collagen type II antibodies. In sum these data suggest that CIKS-mediated signaling should be considered as a therapeutic target for rheumatoid arthritis.

MATERIALS AND METHODS

Mice

CIKS-deficient mice were generated on a 129/SvJ background and backcrossed to the Taconic C57BL/6 (B6) strain for more than 11 generations (17). Wild-type (WT) mice were obtained by interbreeding of heterozygous animals and purchased from Taconic. The generation of 129/B6 Fc γ RIIb deficient mice were described previously (30) and these mice have been backcrossed for 12 generations to the Taconic B6 background (31). Mice were intercrossed to generate compound deficiency in CIKS and Fc γ RIIb as well as control littermates. Mice were bred and housed in National Institute of Allergy and Infectious Diseases (NIAID) facilities, and all experiments were done with approval of the NIAID Animal Care and Use Committee and in accordance with all relevant institutional guidelines.

Collagen-induced arthritis (CIA)

CIA was performed as described (32). In brief, chicken collagen type 2 (CII, 20011) was purchased from Chondrex (Redmond, WA), dissolved in 0.1 mol/l acetic acid (4 mg/ml) and mixed with an equal volume of complete Freund's adjuvant (CFA) (5 mg/ml, 7023; Chondrex, Redmond, WA). Mice were anesthetized with isoflurane before intradermal injection at multiple sites at the base of the tail. Experimental mice received 200 μ g CII and CFA emulsion, and control mice received CFA only. Mice received a booster injection of CFA with CII or of CFA alone 3 weeks after the initial injection. Mice were monitored for arthritis starting 1 week after the booster. The maximal severity of disease was recorded as described (32). Mice that developed more severe arthritis (generally achieving a score of 6 and above between weeks 5-7) had to be euthanized (along with matched controls) as required by the facility veterinarian; the entire study was terminated by 8 weeks. Serum was collected 4 weeks after the first immunization, at a time all animals were still on study.

Collagen antibody-induced arthritis (CAIA)

The arthrogen-CIA monoclonal antibody cocktail (5 distinct clones, 53040) was purchased from Chondrex (Redmond, WA). On day 0, 4 mg of the collagen antibody cocktail was injected intravenously. On day 3, 50 μ g of LPS in sterile PBS was injected intraperitoneally. The control mice only received LPS on day 3. The mice were monitored for clinical signs of arthritis starting 4 days after antibody injection. Severity of arthritis in each limb was scored using the same scale as described for the CIA model above.

Histological analysis

Mice in CIA studies were sacrificed along with matched partners whenever significant signs of arthritis were evident. Joints and bones were fixed with 10% formalin, decalcified with 5% formic acid and embedded in paraffin (21). Biotin anti-mouse neutrophil antibody (7/4,

CL8993B) was purchased from Cedarlane (Burlington, NC). H&E, Safranin O, and neutrophil stainings of tissue sections were performed by Histoserv, Inc (Germantown, MD). The sections were visualized under light microscopy with Olympus BX50 (Olympus, Center Valley, PA) and ProgRes C14 cameras (Jenoptik, Jena, Germany).

Cellular analysis

Spleens were harvested and treated with collagenase D (11088874103; Roche, Indianapolis, IN). Splenocytes were depleted of erythrocytes with ACK lysis buffer and subsequently stained with antibodies to: CD11b (M1/70), Gr-1 (RB6-8C5), CD11c (HL3) (BD Biosciences, San Jose, CA), and F4/80 (BM8) (eBioscience, San Diego, CA). Data were collected with a FACSCanto (BD Biosciences, San Jose, CA) and analyzed using FlowJo software (Tree Star; Ashland, OR).

Quantitative real-time PCR

In brief, RNA was isolated from splenocytes with Trizol (Invitrogen, Carlsbad, CA) and Chloroform. The clear upper layer was collected, precipitated with 70% ethanol and cleaned up with Rneasy kit (Qiagen, Valencia, CA). cDNA synthesis was performed using the iScript cDNA synthesis kit (Biorad, Hercules, CA). The IL-17A and ACTB primers and PCR master mix (4352042) were purchased from Applied Biosystems (Foster city, CA). The real-time quantitative PCR was performed using StepOnePlus (Applied Biosystems, Foster City, CA).

Detection of anti-collagen type 2 specific antibody

Chick collagen type 2 (ELISA grade, 2011) was purchased from Chondrex (Redmond, WA). Plates (Immulon 4HBx; Thermo Scientific, Waltham, MA) were coated with collagen (5 µg/ml) and incubated overnight at 4 °C. Plates were washed 3 times with 0.05% Tween-20 in PBS, blocked with 2% BSA/0.1% Tween-20, incubated for 1 hour at room temperature (RT) and washed 3 more times. The diluted serum was added to the plates, incubated at 4 °C overnight and then plates were washed. Antibodies to IgM, IgG, IgG₁, IgG_{2b}, or IgG_{2c} conjugated with AP (SouthernBiotech, Birmingham, AL) were added to the plates, incubated for 2 hours at RT and plates were washed. The PNPP substrate (SouthernBiotech, Birmingham, AL) was added into the plate and read at 405 nm. The standard plate was coated with anti-mouse Ig(H+L) (human adsorbed) (Southern Biotech, Birmingham, AL) and then serial dilutions of IgM, IgG₁, IgG_{2b}, and IgG_{2c} were added to obtain a standard linear curve.

Joint cell extraction

The isolation of cells from the paws has been described (33). In brief, front and rear paws were harvested and skin was removed. Paws were minced and digested in medium containing 50 µg/ml collagenase VIII (C2139; Sigma, St. Louis, MO), 25 µg/ml Dispase II (04942078001; Roche Diagnostic, Indianapolis, IN), and 20 µg/ml Dnase I (10104159001; Roche Diagnostic, Indianapolis, IN) and incubated at 37 °C for 2 hours with vortexing every 30 minutes. Cells were then passed through a strainer to obtain single cell suspensions and these were subsequently processed for cellular analysis as described for splenocytes above.

RESULTS

CIKS is required for collagen-induced arthritis (CIA) in C57BL/6 mice

To determine whether CIKS is critically involved in arthritis development, we applied the CIA model to CIKS deficient (KO) and wild-type (WT) C57BL/6 (B6) mice. Although DBA mice have traditionally served as the preferred background for CIA, the disease can

also be induced in B6, albeit more modestly (32). Arthritis was induced in WT mice, but could not be induced in CIKS deficient mice as judged by the absence of any swelling of the joints (Figure 1A). The degree of arthritis was determined according to the scale established previously (32). The average maximal arthritis score exceeded '3' in WT mice, but was essentially '0' in CIKS deficient mice (Figure 1B). H&E stained tissue sections from joints of hindpaws showed overt inflammatory cell infiltration and bone erosion in WT, but not CIKS deficient mice (Figure 1C). Safranin O staining revealed disruption of cartilage surfaces in WT, but not CIKS deficient mice (Figure 1D). These results suggest that CIKS is necessary for the development of collagen-induced arthritis in B6 mice. Importantly, the absence of CIKS did not lead to any other obvious phenotypic defects or signs of infections in mice subjected to the CIA studies.

Neutrophil recruitment into arthritic joints and the spleen is CIKS dependent

IL-17A has been identified as a critical contributor in inflammatory arthritis in RA patients and in the CIA mouse model (21,34,35). Neutrophils represent a major portion of the cells infiltrating arthritic joints and they are likely to contribute significantly to overall pathogenesis (36,37). IL-17A is known to be strong effector of neutrophil recruitment into inflammatory sites in several models, including CIA (38-40). To investigate whether neutrophils are recruited into the arthritic joint in CIA in a CIKS dependent manner we performed immunohistochemical analyses of joints. The inflammatory cells infiltrating the arthritic joints of WT mice were mainly neutrophils, as judged by positive staining with the 7/4 antibody (Figure 2A); no such infiltrates could be detected in joints from CIKS deficient mice (Figure 2A). We further analyzed the infiltrates into joints by flow cytometric analyses and confirmed that neutrophils were the major infiltrating cell population, based on high levels of expression of Gr-1, the presence of CD11b and the absence of F4/80 and CD11c markers (data not shown).

In the CIA model collagen type 2 is administered in complete Freund's adjuvant (CFA). Regardless of whether collagen was co-injected, CFA alone already induced a dramatic increase in Gr1^{hi}CD11b⁺ cells in the spleen of WT mice, but only a modest increase in CIKS deficient mice (Figure 2B). Further analysis indicated that the vast majority of Gr1^{hi}CD11b⁺ cells in the spleen also lacked expression of CD11c and F4/80, consistent with primarily neutrophils infiltrating the spleen (Figure 2C). Therefore, CIKS is required for most, though not all of the increase in splenic neutrophils following administration of the CFA adjuvant. To test whether IL-17A could have been responsible for neutrophil recruitment into spleens of CFA-treated mice (with or without collagen), we assessed splenic IL-17A mRNA levels and noted a significant increase of this cytokine following administration of CFA alone (Figure 2D). We conclude that neutrophil recruitment into the arthritic joints following administration of collagen type 2 emulsified with CFA was completely dependent on CIKS-mediated signaling and did not occur with CFA alone, while recruitment of neutrophils into spleens already occurred after administration of CFA alone and was largely, though not totally CIKS dependent.

CIKS contributes to collagen-specific antibody production

Anti-collagen type 2 specific antibodies (CII-Ab) are critical for the pathogenesis of CIA. For example, the susceptibility of mice to develop arthritis has been correlated with the level of CII-Ab (41). Also, transfer of CII-Ab can induce an acute, though transient arthritis-like condition in naïve mice (42). To determine whether CIKS is involved in the generation of CII-Ab, we quantified the levels of the collagen type 2 (CII) specific antibody isotypes by ELISA. Serum antibodies were analyzed at 4 weeks after the first immunization (one week after the boost). The production of CII-specific IgM (Figure 3A) and IgG (Figure 3B) was significantly reduced in CIKS deficient mice. Specifically the production of CII-Ab IgG_{2c}

was most profoundly decreased (Figure 3E), while CII-specific IgG₁ (Figure 3C) and IgG_{2b} (Figure 3D) were more modestly affected. These findings suggest that CIKS contributed to collagen-specific antibody production in B6 mice.

CIKS is not necessary for collagen antibody induced arthritis (CAIA)

The CAIA model has been useful to study mechanisms of inflammatory arthritis in the effector phase, after CII-specific antibodies have been produced (43). Inflammatory antibody-induced arthritis can be readily induced in various strains of mice, including B6 (44). To explore whether CIKS is engaged in the effector phase mediated by antibodies to collagen, we injected a cocktail of several distinct high affinity anti-collagen antibodies along with LPS into WT and CIKS deficient mice as described (43). Inflammatory arthritis appeared to develop in similar fashion in both WT and CIKS deficient mice (Figure 4A). There was no difference in terms of inflammatory cell infiltration and bone erosion (Figure 4B). In addition, cartilage surface was similarly disrupted in both mutant and control mice (Figure 4C). These results indicate that CIKS is not required to induce antibody-induced inflammatory arthritis (the CAIA model).

CIKS is essential for CIA in FcγRIIb deficient mice

FcγRIIb is an inhibitory, ITIM containing receptor, expressed primarily on B cells, but also macrophages, mast cells, and neutrophils (45). It functions to delimit activation in particular of high affinity, autoreactive B cells, thus limiting production of damaging autoantibodies; in the absence of this receptor significant levels of autoantibodies are generated and, over time, a lupus-like condition develops in B6 mice (31). Consistent with this, mice lacking this receptor also exhibited exacerbated arthritis in the CIA model, presumably due to increased levels of high-affinity autoantibodies (29). We also tested the relevance of CIKS for CIA pathology in the absence of FcγRIIb, given the relatively modest arthritis scores achievable in WT B6 mice. We carried out the CIA studies in 6-8 week old CIKS sufficient and deficient mice lacking FcγRIIb; we used such relatively young mice in order to avoid possible complications due to the lupus-like condition that develops in much older FcγRIIb-deficient mice. Despite increased CIA scores in FcγRIIb-deficient mice (as compared to WT), arthritis development was still completely prevented if mice were also deficient in CIKS (Figure 5A, 5B); no inflammatory cells were evident in joints (Figure 5C) and bone surfaces and articular cartilage were fully preserved (Figure 5D). These effects indicate that CIKS is absolutely required for arthritis development in the CIA model in FcγRIIb-deficient mice.

CIKS contributes significantly to the generation of collagen-specific IgG antibodies in FcγRIIb deficient mice

In the absence of FcγRIIb, autoreactive B cells are more readily activated and differentiate into IgG-producing cells that generate significant levels of autoantibodies over time (31). To determine whether CIKS has a similar role in generation of CII-Ab in mice lacking FcγRIIb as it does in FcγRIIb-sufficient B6 mice (see Figure 3), we determined the isotypes and levels of CII-Ab after CIA in CIKS sufficient and CIKS deficient mice in the absence of FcγRIIb. When compared to the studies in B6 mice above, loss of CIKS only modestly reduced the levels of CII-Ab of the IgM subclass in FcγRIIb deficient mice (Figure 6A). However, the levels of IgG CII-Ab were markedly reduced in the compound deficient mice when compared to FcγRIIb singly deficient mice (Figure 6B); furthermore, the reduction caused by the absence of CIKS was more pronounced on this background than what was observed in the FcγRIIb sufficient background (see Figure 3). Levels of all IgG subclasses of the CII-Ab tested were significantly decreased (IgG₁, IgG_{2b}, IgG_{2c} [Figures 6C-E] and IgG₃ [not shown]). These results suggest that CIKS-mediated signaling may have

contributed to IgG isotype class switching and/or IgG production in response to CIA in Fc γ RIIb deficient mice.

DISCUSSION

The various physiologic roles of the members of IL-17 cytokine family in both health and disease remain to be fully explored. Based on present understanding some of the members may have distinct and possibly even opposing activities, such as IL-25 and IL-17A, while others may have redundant activities. IL-17A has been clearly recognized for its pathogenic role in CIA (21,34,46). IL-17A deficient mice develop a significantly ameliorated form of arthritis compared to wild-type mice, including reduced pathology upon histological examination; however loss of this cytokine does not fully abrogate arthritis pathology (21). In addition to IL-17A, recently emerging data suggest that IL-17B and IL-17C and, to some extent, even IL-17F, may contribute to the development of inflammatory arthritis, suggesting possibly redundant functions of these IL-17 family members in the context of joint pathology (25,26). These findings could explain why arthritis development is not fully abrogated in the absence of IL-17A alone.

The adaptor protein CIKS (a.k.a. Act1) likely mediates signaling by all members of the IL-17 cytokine family, although this remains to be formally demonstrated. CIKS has been shown to be absolutely required for signaling by IL-17A, IL-17F as well as IL-25, the most divergent member of the IL-17 cytokine family (17). We demonstrate here that collagen-induced arthritis (CIA) was completely abrogated in the absence of CIKS. The protective effect afforded to mice lacking CIKS appeared more pronounced than that afforded to mice lacking IL-17A (21). The total prevention of any pathology may have been the result of blocking signaling by all members of the IL-17 cytokine family that contribute to arthritis pathology, not just IL-17A.

Of note, the absence of CIKS did not enhance antibody formation as might have been predicted by data obtained with one of the two CIKS/Act1 deficient mouse models (15) (see Introduction). Here the absence of CIKS did in fact cause a reduction of autoantibodies in the context of the CIA studies. Loss of CIKS also did not indirectly enhance arthritis by interfering with possible anti-Th17 effects of IL-25, assuming IL-25 is generated in the CIA model.

How might the IL-17 cytokines function to promote arthritis? A hallmark feature of IL-17A and possibly other members of the family is the recruitment of neutrophils (38-40). Neutrophils also represented the dominant inflammatory cell infiltrate in arthritic joints in the CIA model here, and this infiltration was totally dependent on CIKS. The recruitment of large numbers of neutrophils into joints could be due to collagen-specific Th17 cells or other IL-17 family cytokine-producing cells at these anatomical sites (40). The continued presence of neutrophils and their products presumably cause tissue damage in joints, although IL-17-type cytokines are likely to have additional direct and indirect effects that contribute to pathology as well (2,34,39). It is interesting to note that neutrophil recruitment into spleens, though importantly not into joints, was already observed in response to administration of the adjuvant CFA without CII. This recruitment was also largely, though not completely dependent on CIKS and consistent with the production of IL-17A in spleens of CFA treated mice. The CFA adjuvant may favor production of IL-17 type cytokines, which in turn may promote arthritis if collagen is administered together with this adjuvant. This interpretation is also consistent with the ability of the CFA adjuvant alone to induce arthritis in mice lacking the IFN γ R (47).

Our findings suggest that CIKS-dependent IL-17 type cytokine signaling also contributed to the production of collagen-specific antibodies. Immunization with collagen and CFA induced antibodies of mainly the IgG₁, IgG_{2b}, and IgG_{2c} subclasses in C57BL/6 mice. CIKS-mediated signaling significantly enhanced levels of IgM and especially of IgG_{2c} antibodies, which may be critical for development of arthritis in C57BL/6 mice (Collagen-specific IgG_{2a} has been shown to correlate with the severity of arthritis and the clinical onset of disease in DBA mice (48); C57BL/6 produce IgG_{2c} instead of IgG_{2a}).

CIKS also profoundly promoted the production of CII-specific IgG_{2b} antibodies in FcγRIIb-deficient mice. In these mice CIKS played an even more important role in the production of CII-specific antibodies when compared to FcγRIIb-sufficient mice. CIKS-mediated signaling significantly boosted the production of all IgG subclasses tested, including IgG₁, IgG_{2b}, IgG_{2c} (as shown) as well as IgG₃ (not shown). The increased susceptibility of FcγRIIb-deficient mice to CIA has been proposed to be due to the lower activation threshold of autoreactive (collagen-specific) B cells to develop into autoantibody producing B cells (29,30). The FcγRIIb-deficient mice used for the CIA studies were young mice (6-8 weeks) that had not yet spontaneously developed autoantibodies; this allowed us to study the role of CIKS in a background that was more susceptible to CIA without complications that might arise from other autoimmune phenotypes. Still, CIKS was absolutely required for the development of any CIA-associated pathology and it had a marked role in the production of autoantibodies.

The findings with mice sufficient and deficient in FcγRIIb indicate that CIKS is involved in CII-Ab production in the CIA model, but it remains to be fully explored at what stage CIKS and presumably IL-17 family cytokines play a role. In this regard it is noteworthy that IL-17A has been proposed to contribute to the survival and proliferation of human B cells and their differentiation into immunoglobulin-secreting cells in the context of lupus (49). Furthermore, IL-17A has also been reported to promote autoreactive germinal center development in autoimmune BXD2 mice (50).

CIKS was not required for the development of acute inflammatory arthritis in the antibody transfer model (CAIA). Therefore CIKS is not likely to be significantly involved in the antibody-induced effector phase of arthritis. However, this model depends on massive administration of high-affinity collagen-specific antibodies. The collagen-specific antibodies produced in the CIA model may not by themselves be sufficient to induce full arthritis without additional contributions by IL-17-type cytokines during the effector phase; furthermore, continued production of high-affinity autoantibodies by B cells in the CIA model may depend on signaling by IL-17 family cytokines.

In sum our data demonstrate that the CIKS adaptor protein is absolutely required for the development of joint pathology in the CIA model; CIKS-mediated signaling is responsible for inflammatory cell infiltration into joints, bone erosion and cartilage damage and it significantly contributes to CII-specific antibody production. The total dependence of joint pathology on CIKS-mediated signaling may reflect multiple contributions of IL-17 family cytokines to the development of arthritis. As such, CIKS and the signaling pathways it regulates may provide highly sensitive targets for therapeutic intervention in the development of rheumatoid arthritis.

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Figure 1.

CIKS is required for collagen-induced arthritis (CIA) in C57BL/6 mice (WT). A, Examples of paws from WT and CIKS-deficient mice (CIKS [KO]) after CIA, representative of 4 independent experiments involving multiple mice per experiment. B, Clinical scores of CIA mice reflecting maximal scores prior to euthanization; each individual dot represents one animal (WT (N=27), CIKS[KO] (N=18), p value=0.001 (T-test)). C and D, Representative examples of histological examinations. C, H&E staining of the joints showing inflammatory cell infiltration, joint space narrowing and bone erosion (arrowheads) in WT mice. D, Safranin O staining showing disruption and irregularity of cartilage surface in WT mice. Histological slides illustrated at 10X magnification. Histological analysis was performed between 2-3 weeks after the booster injection.

Figure 2. Neutrophil recruitment into arthritic joints and spleen is CIKS dependent. A, Immunohistochemistry with the neutrophil antibody (7/4) was performed with joint sections from WT and CIKS[KO] mice subjected to the CIA protocol. Neutrophil staining (brown) was detected in WT mice, but not in CIKS[KO] mice. Histological slides illustrated at 10X magnification. Histological analysis was performed between 2-3 weeks after the booster injection. B and C, Splenocytes from WT and CIKS[KO] mice after treatment with CFA, CIA (CII and CFA) for 1-2 weeks after the booster injection or no treatment were stained with CD11b, Gr-1, CD11c, and F4/80 antibodies and analyzed by flow cytometry. B, Shows the percentage of CD11b⁺Gr-1^{hi} cells. C, Analysis for F4/80 and CD11c gated on CD11b⁺Gr-1^{hi} cells. Data is representative of at least 3 independent experiments. D, Quantitative PCR analysis for IL-17 expression in splenocytes isolated from CFA or untreated mice (WT, black bar; CIKS[KO], white bar), 2 weeks after the booster injection. Error bar indicates SEM; p value: * < 0.05; ** < 0.01 (T-test). Data are from 2 independent experiments.

Figure 3.

CIKS contributes to collagen-specific antibody production. Anti-collagen type 2 (CII) specific antibodies were detected in the serum of WT (black bar) and CIKS[KO] (white bar) mice 1 week after the booster injection by ELISA. A, Serum dilution of 1:100 was used to detect CII-IgM. B-E, Serum dilution of 1:500 was used to detect CII-IgG (B), CII-IgG₁ (C), CII-IgG_{2b} (D), and CII-IgG_{2c} (E). Data collected from WT (N=13, CFA and N=20, CII/CFA) and CIKS[KO] (N=10, CFA, and N=14, CII/CFA). Error bar indicates SEM, p value; * < 0.05; ** < 0.01 (T-test).

Figure 4. CIKS is not necessary for collagen antibody-induced arthritis (CAIA). A, Clinical scores of CAIA mice reflecting maximal scores prior to euthanization; each individual dot represents one animal (WT (N=6), CIKS[KO] (N=7), p value>0.05 (T-test)). B and C, Representative examples of histological examination of joint sections. B, H&E staining of joints showing inflammatory cell infiltration, and bone erosion (arrowheads) in both WT and CIKS[KO] mice. C, Safranin O staining showing disruption and irregularity of cartilage surface in both WT and CIKS[KO] mice. Histological slides illustrated at 10X magnification. Histological analysis was performed between 7-10 days after injection of antibodies.

Figure 6.

CIKS contributes to IgG isotype collagen-specific antibody production in FcγRIIb deficient mice. Anti-collagen type 2 (CII) specific antibodies were detected in the serum of FcγRIIb[KO] (black bar) and FcγRIIb[KO].CIKS[KO] (white bar) mice 1 week after the booster injection by ELISA. A, Serum dilution of 1:100 was used to detect CII-IgM. B-E, Serum dilution of 1:500 was used to detect CII-IgG (B), CII-IgG₁ (C), CII-IgG_{2b} (D), and CII-IgG_{2c} (E). Data collected from FcγRIIb[KO] (N=8, CFA and N=13, CII/CFA) and FcγRIIb[KO].CIKS[KO] (N=6, CFA, and N=6, CII/CFA). Error bar indicates SEM, p value; * <0.05; ** <0.01 (T-test).