Protective Role of Collectin 11 in a Mouse Model of Rheumatoid Arthritis

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Objective. Collectin 11 (CL-11) is a soluble C-type lectin, a mediator of innate immunity. Its role in autoimmune disorders is unknown. We undertook this study to determine the role of CL-11 in a mouse model of rheumatoid arthritis (RA).

Methods. A murine collagen-induced arthritis (CIA) model was used and combined two approaches, including gene deletion of Colec11 and treatment with recombinant CL-11 (rCL-11). Joint inflammation and tissue destruction, circulating levels of inflammatory cytokines, and adaptive immune responses were assessed in mice with CIA. Splenic CD11c+ cells were used to examine the influence of CL-11 on antigen-presenting cell (APC) function. Serum CL-11 levels in RA patients were also examined.

Results. Colec11−/− mice developed more severe arthritis than wild-type mice, as determined by disease incidence, clinical arthritis scores, and histopathology (P < 0.05). Disease severity was associated with significantly enhanced APC activation, Th1/Th17 responses, pathogenic IgG2a production and joint inflammation, as well as elevated circulating levels of inflammatory cytokines. In vitro analysis of CD11c+ cells revealed that CL-11 is critical for suppression of APC activation and function. Pharmacologic treatment of mice with rCL-11 reduced the severity of CIA in mice. Analysis of human blood samples revealed that serum CL-11 levels were lower in RA patients (n = 51) compared to healthy controls (n = 53). Reduction in serum CL-11 was inversely associated with the Disease Activity Score in 28 joints, erythrocyte sedimentation rate, and C-reactive protein level (P < 0.05).

Conclusion. Our findings demonstrate a novel role of CL-11 in protection against RA, suggesting that the underlying mechanism involves suppression of APC activation and subsequent T cell responses.

INTRODUCTION

Collectins are a group of soluble C-type lectins; mannose-binding lectin (MBL), collectin 10, and lung surfactant proteins (surfactant proteins A and D) are well-known members of the group. They function as pattern-recognition receptors that bind to carbohydrates or carbohydrate moieties on the surface of pathogens and host cells, and they play a role in modulation of cellular processes in addition to participating in host defense (1–3). Collectin 11 (CL-11, also known as CL-K1) is another member of the collectin family, displaying structural similarities with other collectins. However, it displays some unique characteristics such as having a wide tissue distribution, a relatively low serum concentration, and binding a wide range of ligands (4–7). These

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characteristics suggest that CL-11 may be involved in a broad range of cellular processes, and local production of CL-11 may play an important role in these cellular processes. CL-11 is highly conserved among different species; humans and mice show 92% homology at the amino acid level (5). Functional protein is derived from the COLEC11 gene on chromosome 2 in humans and chromosome 12 in mice. CL-11 has been shown to have an important function in embryogenesis and host defense and to mediate the pathogenesis of renal ischemia reperfusion injury (8–11). Additionally, in a recent study in retinal pigment epithelial cells, we found that CL-11 up-regulates interleukin-10 (IL-10) but down-regulates IL-6 production by the cells, suggesting a critical role of CL-11 in immune regulation (12). Taken together, findings from these studies suggest that CL-11 is a multifunctional molecule that can participate in diverse biologic processes. To date, the functional roles of CL-11 have been described in either nonimmune or innate immune settings. It is unknown whether CL-11 plays an important role in adaptive immunity.

Rheumatoid arthritis (RA) is a chronic, progressive inflammatory autoimmune disease, manifesting both joint and systemic effects. It is characterized by general synovial inflammation, cartilage destruction and bone erosion, with accompanying elevation of circulating autoantibodies and systemic inflammation (13). The pathogenesis of RA is not fully understood, but it is thought that genetic susceptibility and environmental factors (e.g., smoking, infections) trigger abnormal autoimmune responses, which involve both innate and adaptive immune responses (14). Previous studies have suggested that the pathogenic process in RA involves several stages. These stages include the following: 1) the induction of adaptive immune responses that lead to T cell and B cell activation and autoantibody production (the initial phase), 2) subsequently, the occurrence of synovial inflammation, with inflammatory cell infiltration, cell activation, and increase in cytokine production (the effector phase), and 3) eventually, the inflammation is converted to a chronic process which leads to the release of cytokines, proteases, and other mediators that cause tissue destruction (the chronic phase) (15).

The dysregulation of adaptive immune responses is thought to play a significant role in the pathogenesis of RA (13). Clinical studies have shown that multiple autoantibodies—due to epitope spreading—can be detected in the serum and synovium of RA patients (16), and T cell costimulation blockade produces significant clinical and functional benefits in patients who have had an inadequate response to anti–tumor necrosis factor (anti–TNF) therapy (17). Experimental studies in murine models of RA have further demonstrated the importance of CD4+ T cells, specifically Th17 cells in the pathogenesis of RA, particularly in the initial phase of the autoimmune reaction and in inducing local inflammation in the joints (18). Despite the major role of the adaptive immune response, innate immune responses could also have an important function in the initiation of RA. Adaptive immunity effectors mechanisms, such as the action of Th17 cells, require the participation of innate cells and cytokine/chemokines. The inflammatory environment plays a key role in shaping adaptive immune responses. Indeed, a large number of studies have shown that innate immune responses have an important function in RA. Proinflammatory cytokine/chemokine production, inflammatory cell infiltration, and complement activation in synovium have been implicated in tissue inflammation and bone destruction (19–21). However, most of these studies have focused on the role of innate immune responses in the pathogenesis of effector and chronic phases. Little is known about whether or how the innate immune system can act in concert with adaptive immune responses and impinge on the initiation of RA.

Given that CL-11 is a pattern-recognition molecule with multiple potential functions that also possesses immune regulatory properties, we hypothesized that CL-11 might play an important role in shaping adaptive immunity, thereby influencing the development of RA. In the present study, we investigated this hypothesis. A murine model of RA induced by collagen, which included both deletion of the Colec11 gene and treatment with recombinant CL-11 (rCL-11), was used to determine the role of CL-11 in disease development and progression. Joint inflammation and tissue destruction, circulating levels of inflammatory cytokines, and adaptive immune responses were assessed following collagen immunization. Splenic CD11c+ cells were used to examine the influence of CL-11 on antigen-presenting cell (APC) cytokine secretion and function in T cell stimulation. Additionally, we assessed the clinical relevance of CL-11 in RA by measuring serum CL-11 levels in RA patients.

PATIENTS AND METHODS

Mice. Homozygous Colec11−/− mice on a C57BL/6 background (22) were obtained from Mutant Mouse Resource and Research Centers (University of California, Davis) and were back-crossed to the C57BL/6 strain for least 8 generations. Wild-type (WT) littermates were used as controls. Male mice (10–14 weeks of age) were used in all experiments unless specified otherwise. All mice were maintained in specific pathogen–free conditions on a 12-hour reversed light/dark cycle. The Ethics Review Committee for Animal Experimentation at Xi’an Jiaotong University approved and oversaw all mouse experiments.

Patients. A total of 51 patients diagnosed with RA and 53 healthy donors were enrolled. The study was approved by the Ethics Committee Board of the No. 5 Hospital of Xi’an and the Second Affiliated Hospital of Xi’an Jiaotong University. Informed consent was obtained from each participant according to the regulations of our institutional ethics committee. The clinical characteristics of the patients are provided in Supplementary Table 1 (available on the Arthritis & Rheumatology website at http://onlinelibrary.wiley.com/doi/10.1002/art.41696/abstract).
Induction of collagen-induced arthritis (CIA) and clinical evaluation. Arthritis was induced in WT mice and Colec11−/− mice or rCL-11–treated mice using a previously described protocol in C57BL/6 mice (23) (Supplementary Figure 1A, http://onlinelibrary.wiley.com/doi/10.1002/art.41696/abstract). Clinical arthritis was evaluated using a previously described scoring system (24,25). The incidence of arthritic paws was defined as the occurrence of inflamed paws with a clinical arthritis score of ≥2 (24) by 2 independent observers (NW, WW) in a blinded manner. Serum cytokine levels, C3a/C5a, and collagen-specific IgG/IgG2a were measured by enzyme-linked immunosorbent assay (ELISA). Histopathologic changes were assessed using hematoxylin and eosin (H&E)–stained and toluidine blue–stained sections by 2 independent observers in a blinded manner. Cellular infiltration in joint tissue was assessed by immunohistochemistry. Tissue inflammation was assessed by reverse transcriptase–quantitative polymerase chain reaction (RT-qPCR) for key inflammatory mediators. Splenocytes isolated from WT mice with CIA and Colec11−/− mice with CIA were used to evaluate frequencies of CD4+ T cells, CD8+ T cells, and B cells and for detection of interferon-γ (IFNγ)– or IL-17A–producing cells by flow cytometry analysis.

In vitro experiments. Splenic CD11c+ cells from WT mice and Colec11−/− mice were prepared and used to investigate the impact of CL-11 on APC cytokine secretion and capacity to stimulate T cells, by flow cytometry and ELISA.

Figure 1. Colec11−/− mice develop more severe collagen-induced arthritis (CIA). A–C, Incidence of arthritis (A), clinical arthritis scores from day 24 to day 42 (B), and clinical arthritis scores on day 42 (C) in the paws of wild-type (WT) mice (n = 40) and Colec11−/− mice (n = 36). D, Representative microscopic images of hematoxylin and eosin (H&E) and toluidine blue (T-blue) staining in knee joint sections on day 42. Arrows indicate lesions and abnormalities in the joint. Bars = 200 μm. The bottom panels show higher-magnification images of the boxed regions in the panels above. E, Separate histologic scores (n = 8 mice per group). F, Representative images of immunohistochemical staining for F4/80 (macrophages) and Ly6B.2 (neutrophils) in knee joint sections from WT and Colec11−/− mice with CIA on day 42 (n = 3 mice/group). Arrows indicate the positively stained cells. Bars = 20 μm. The bottom panels show higher-magnification images of the boxed regions in the panels above. B = bone; SM = synovial membrane; JS = joint surface. Symbols represent individual mice; bars show the mean ± SD. * = P < 0.05; ** = P < 0.01; *** = P < 0.001; **** = P < 0.0001, by chi-square test (A), two-way analysis of variance with multiple comparisons test (B and E), or unpaired t-test (C).
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Statistics. Data are presented as the mean ± SD. Unpaired t-test was used to compare 2 groups. Paired t-test was used to compare the means of matched pairs. Analysis of variance was used to compare the means of ≥2 independent groups. Pearson’s correlation coefficient was used to measure the degree of relationship between the 2 groups of data. All analyses were performed using GraphPad Prism 7 software. P values less than 0.05 (2-tailed) were considered significant.

Additional details. Reagents and more detailed methods for clinical arthritis scoring, histopathology of knee joints, immunohistochemistry, detection of CL-11, CD11c, and CD4 in the spleen, ex vivo analysis of T cell and B cell responses in mice with CIA, isolation of CD11c+ cells and CD4+ T cells, in vitro analysis of antigen-specific T cell responses, flow cytometry analysis, RT-qPCR, and ELISA are described in Supplementary Methods (http://onlinelibrary.wiley.com/doi/10.1002/art.41696/abstract).

RESULTS

More severe CIA development in Colec11−/− mice. We induced arthritis in Colec11−/− mice and WT mice to determine the role of CL-11 in this model of RA. The incidence and severity of arthritis were assessed visually beginning 24 days after the first immunization (3 days after the second booster immunization), at intervals of 2 days, for up to 42 days. Colec11−/− mice displayed a higher incidence of arthritis than WT mice (Figure 1A). Colec11−/− mice also developed more severe arthritis with significantly higher clinical scores than WT mice across all time points (Figures 1B and C). H&E and toluidine blue staining showed that Colec11−/− mice with CIA exhibited more severe synovial inflammation (i.e., synovial edema and hyperplasia, inflammatory infiltrates in synovium, pannus formation and its invasion of articular cartilage and bone), cartilage damage (loss of proteoglycan on the surface), and bone erosion, compared to WT mice with CIA (Figures 1D and E). Immunohistochemistry showed that the number of macrophages (F4/80+) and neutrophils (Ly6B.2+) in knee joint tissue was higher in Colec11−/− mice with CIA compared to WT mice with CIA (Figure 1F). Taken together, these results demonstrate that CL-11 deficiency promotes more severe arthritis.

Increased joint inflammation and elevated circulating levels of inflammatory cytokines in Colec11−/− mice with CIA. Joint inflammation was analyzed by examining tissue messenger RNA (mRNA) levels of key proinflammatory cytokines by RT-qPCR. Colec11−/− mice with CIA had significantly higher levels of mRNA for Tnf, Il1b, Il6, Ccl8, Ccl2, and Rankl, compared to WT mice with CIA (Figure 2A). Circulating levels of inflammatory cytokines in mice with CIA were analyzed by ELISA. Colec11−/− mice with CIA had significantly higher serum levels of TNF, IL-6, and CCL2, but lower levels of IL-10, compared to WT mice (Figure 2B). These results demonstrate that CL-11 deficiency leads to increased joint inflammation and systemic inflammatory responses. There was no significant difference in basal levels of inflammatory cytokines between naive Colec11−/− mice and WT mice (Supplementary Figure 2A, http://onlinelibrary.wiley.com/doi/10.1002/art.41696/abstract), indicating that the observed differences in cytokine production resulted from differential inflammatory responses to collagen antigen.

Enhanced adaptive immune responses following collagen immunization in Colec11−/− mice. Adaptive immune responses contribute to the pathogenesis of RA in humans and CIA in mice. We therefore assessed the impact of CL-11 deficiency on adaptive immune responses following collagen immunization. We examined serum levels of T helper cell index cytokines and collagen-specific antibodies in WT and Colec11−/− mice with CIA on day 42 postinduction. Colec11−/− mice with CIA had significantly higher serum levels of IFNγ and IL-17A, as well as collagen-specific total IgG and IgG2a, compared to WT mice with CIA (Figures 2B and C). These findings indicate that CL-11 deficiency leads to enhanced adaptive immune responses following antigen immunization.

CL-11 deficiency-dependent enhancement of adaptive immune responses was further evaluated by analyzing T cell and B cell activation in WT and Colec11−/− mice with CIA on days 25 and 42 from isolated splenocytes. Colec11−/− mice with CIA had significantly higher frequencies of splenic CD4+ T cells and CD8+ T cells on day 25 compared to WT mice with CIA. However, the frequency of CD19+ B cells was lower in Colec11−/− mice with CIA (potentially reflecting a relative change in the CD45+ compartment) (Figure 2D). Frequencies of regulatory T cells in the spleen were comparable between the WT and Colec11−/− mice with CIA (Supplementary Figure 3, http://onlinelibrary.wiley.com/doi/10.1002/art.41696/abstract). Next, we analyzed IFNγ- and IL-17A–producing CD4+ T cells in splenocytes isolated from WT and Colec11−/− mice with CIA. Compared to WT mice with CIA, Colec11−/− mice with CIA had a significantly higher percentage of IFNy+ and IL-17A+ cells in the CD4+ T cell population on days 25 and 42 (Figure 2E). There was no statistically significant difference in the basal levels of T cell and B cell activities, or serum cytokines, between naive Colec11−/− mice and WT mice (Supplementary Figures 2B and C).

APC maturation/activation is critically important for the initiation and direction of T cell responses. We therefore investigated whether CL-11 deficiency can impact APC maturation/activation. We analyzed CD11c+ cells from the draining lymph nodes of WT and Colec11−/− mice 24 hours after collagen immunization. Lymph node CD11c+ cells from Colec11−/− mice displayed higher levels of surface major histocompatibility complex (MHC) class II, CD86, and CD40 than the cells from WT mice (Figure 2F).

Taken together, these results indicate that ablation of CL-11 leads to enhanced adaptive immune responses following the collagen immunization.
Impact of CL-11 deficiency on APC function. Impact of CL-11 deficiency on APC function was evaluated using both splenic CD11c+ cell culture and a coculture model for measuring antigen-specific T cell responses. Isolated splenic CD11c+ cells (from WT and Colec11−/− mice) were further cultured for 24 hours, and inflammatory cytokine secretion was assessed. Compared to splenic CD11c+ cells from WT mice, splenic CD11c+ cells from Colec11−/− mice produced higher levels of proinflammatory cytokines (i.e., TNF, IL-12) but lower levels of IL-10 (Figure 3A). Isolated splenic CD11c+ cells (from WT and Colec11−/− mice) were also cocultured with syngeneic splenic CD4+ T cells (from collagen antigen-primed C57BL/6) in the presence of collagen antigen for
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up to 72 hours, and T cell responses were analyzed by assessing IFNγ- or IL-17A–producing CD4+ T cells and cytokine secretion. Compared to splenic CD11c+ cells from WT mice, splenic CD11c+ cells from Colec11−/− mice induced higher T cell responses, as evidenced by an increase in IFNγ- and IL-17A–producing CD4+ T cells (Figure 3B). In addition, higher levels of IL-2, IFNγ, IL-17A, IL-12, and TNF were observed in coculture supernatants (Figures 3C and D). These results, taken together with in vivo observations about lymph node CD11c+ cells, indicate that CL-11 deficiency leads to enhanced APC activation and function.

Figure 3. Impact of collectin 11 (CL-11) deficiency on function. A, Cytokine levels in 24-hour culture supernatants of splenic CD11c+ cells derived from WT or Colec11−/− mice in the presence of lipopolysaccharide (10 ng/ml) were determined by enzyme-linked immunosorbent assay (ELISA). B, Percentage of IFNγ+ and IL-17A+ cells in the CD4+ T cell population in the coculture of primed CD4+ T cells and splenic CD11c+ cells derived from WT or Colec11−/− mice in the presence of collagen for 72 hours was determined by intracellular staining/flow cytometry analysis. C and D, Cytokine levels in 48-hour or 72-hour coculture supernatants were determined by ELISA. E, Immunohistochemical staining for CL-11 (red), CD11c (green), CD4 (white), and nuclei (DAPI; blue) was performed on the spleen section from a mouse 24 hours after the booster immunization. Top, Positive staining for CL-11 was detected in the marginal zone (MZ) and T cell zone (TZ) with less intense staining in the germinal center (GC). Bar = 50 μm. Bottom, Higher-magnification images corresponding to the boxed regions in the top panel show that a majority of CD11c+ cells were positively stained for CL-11 in the marginal zone (indicated by arrows) (image 1); CD4+ T cells were rarely positively stained for CL-11 in the T cell zone (indicated by arrows) (image 2). Bars = 10 μm. * = P < 0.05; ** = P < 0.01; *** = P < 0.001, by paired t-test (n = 3–4 independent experiments). See Figure 2 for other definitions.
Next, we assessed the possibility of an interaction of CL-11 with APCs and T cells in the spleen. Immunohistochemical staining for CL-11, CD11c, and CD4 was performed on spleen sections (24 hours after the booster immunization), to examine the distribution and localization of CL-11. CL-11 was clearly detected in the spleen and was mainly distributed in the marginal and T cell zones and less in the germinal center. A majority of the CD11c+ cells were positively stained for CL-11, and CD4+ T cells were rarely positively stained for CL-11 (Figure 3E). Thus, abundant distribution of CL-11 in the marginal and T cell zones, as well as colonization of CL-11 with CD11c+ cells, support the notion of an interaction of CL-11 with APCs and possibly T cells.

Pharmacologic treatment of mice with rCL-11. Having demonstrated the role of CL-11 in protection against CIA by using Colec11−/− mice, we next explored whether pharmacologic treatment of mice with rCL-11 could reduce CIA. Two treatment protocols were employed, including early treatment (starting 24 hours before the induction of CIA) and late treatment (starting on the day of the booster immunization) (Supplementary Figures 1B and C, http://onlinelibrary.wiley.com/doi/10.1002/art.41696/abstract). Severity of arthritis, serum levels of proinflammatory cytokines, and antigen specific IgG levels were assessed as described in Figure 1. Compared to the control group, mice receiving early treatment with rCL-11 developed less severe CIA, as evidenced by reduced arthritis scores and reduced serum levels of IFNγ, IL-17A, TNF, and type II collagen–specific IgG/IgG2a. In contrast, serum levels of IL-10 were higher in the rCL-11 treatment group compared to the control group (Figures 4A–C). Mice receiving late treatment with rCL-11 also displayed reduced arthritis scores and type II collagen–specific IgG2a levels (but higher IL-10 levels) compared to the control group, though the differences were small (Figures 4D–F). Other pathologic parameters, including serum cytokines (TNF, IFNγ, IL-17A) and type II collagen–specific total IgG levels, were not statistically different between the 2 groups, although there was a trend toward a reduction in the rCL-11–treated group.

Figure 4. Pharmacologic treatment of mice with recombinant collectin 11 (rCL-11). A–C, Administration of rCL-11 or bovine serum albumin (BSA) (used as control [ctrl]) to WT mice started 24 hours before the first collagen immunization. Clinical arthritis scores from day 24 to day 42 (A), serum cytokine levels (B), and serum type II collagen–specific total IgG/IgG2a levels (C) (n = 10 mice per group) are shown. D–F, Administration of rCL-11 or BSA to WT mice started 21 days after the first collagen immunization. Clinical arthritis scores from day 24 to day 42 (D), serum cytokine levels (E), and serum type II collagen–specific total IgG/IgG2a levels (F) (n = 12 mice per group) are shown. In B, C, E, and F, symbols represent individual mice; bars show the mean ± SD. The horizontal dotted lines show the cytokine levels in the serum from normal mice. * = P < 0.05; ** = P < 0.01; *** = P < 0.001, by two-way analysis of variance with a test for multiple comparisons (A and D) or unpaired t-test (B, C, E, and F). NS = not significant (see Figure 2 for other definitions).
(Figures 4D–F). These findings indicate that pharmacologic treatment of mice with rCL-11 reduces CIA, with the early treatment protocol providing improved protection compared to the late treatment protocol.

Clinical relevance of CL-11 in RA patients. We explored the clinical relevance of CL-11 in RA patients. We analyzed the serum levels of CL-11 in RA patients (n = 51) and healthy controls (n = 53) by ELISA. CL-11 levels in RA patients were significantly lower compared to controls (Figure 5A). We also performed correlation analysis in RA patient samples to assess the strength of the relationship between CL-11 levels and the Disease Activity Score in 28 joints (DAS28) (26) or the levels of several inflammatory and immunologic molecules that are usually present in a person with RA. CL-11 levels were negatively correlated with the DAS28, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) level (Figure 5B). There was no apparent correlation between CL-11 levels and either anti–cyclic citrullinated peptide antibody (anti-CCP) or rheumatoid factor levels (Figure 5C). These observations indicate a negative relationship between the serum CL-11 levels and the severity of RA as well as key inflammatory markers (ESR, CRP level).

**DISCUSSION**

Although it is well recognized that both innate and adaptive immune responses contribute to the pathogenesis of autoimmune diseases such as RA, there is relatively less known about the regulation at the interface of the 2 arms of the immune system, particularly in the early phase of the disease. In this study, we have demonstrated a novel role of CL-11 as an important link between innate and adaptive immune responses, whereby CL-11 mediates suppression of APC activation/function and subsequent T cell responses, ultimately limiting inflammatory responses and conferring protection against CIA.

We used a murine CIA model, which included mice lacking the *Colec11* gene and WT mice that were administered pharmacologic rCL-11, in order to determine the role of CL-11 in CIA. The results of our in vivo experiments, in which multiple parameters (i.e., clinical arthritis scores, joint tissue inflammation/destruction, serum levels of proinflammatory cytokines and antigen-specific antibodies) were measured, clearly show that CL-11 is required for suppression of CIA, as mice lacking CL-11 developed more severe CIA. These observations support a protective role of CL-11 in the development and progression of destructive arthritis.

In addition to demonstrating the protective role of CL-11 in CIA, we investigated how CL-11 confers this protection. A key finding in this regard is that *Colec11<sup>−/−</sup>* mice exhibited markedly enhanced Th1 and Th17 responses and antigen-specific antibody production. This suggests that CL-11 has a suppressive effect on adaptive immune responses. To elucidate potential mechanisms of CL-11–dependent suppression of the adaptive immune response, we identified CL-11 as critical for suppression of APC activation/function. This conclusion was supported by several findings from in vivo and ex vivo analyses of APCs. The first finding was that CD11c<sup>+</sup> cells from draining lymph nodes of immunized
Colec11−/− mice displayed a more activated phenotype, with enhanced expression of MHC class II and costimulatory molecules. The second observation was that splenic CD11c+ cells from Colec11−/− mice secreted more proinflammatory cytokines. Additionally, splenic CD11c+ cells from Colec11−/− mice, when cocultured with syngeneic CD4+ T cells in the presence of collagen antigen, induced enhanced antigen specific T cell responses.

An important question that arises from our observation of the impact of CL-11 deficiency on CD11c+ cell activation is how the absence of CL-11 leads to overactivation of CD11c+ cells. One possibility is that extracellular CL-11 released from dendritic cells (DCs) and possible bystander cells, as well as from the circulation, could establish suppressive effects on cell maturation/activation. This idea could be supported by our 2 observations. First, in the present study, we observed abundant distribution of CL-11 in the marginal and T cell zones, and colocalization of CL-11 with CD11c+ cells. Second, in a previous study, we showed that bone marrow CD11c+ cells can synthesize and secrete CL-11, and the addition of rCL-11 to bone marrow CD11c+ cell culture medium inhibited proinflammatory cytokine production in these cells (27). It is known that CL-11 has a wide spectrum of carbohydrate ligands. Notably, CL-11 has been shown to bind mannose residues on retinal epithelial cells and fibroblasts, thereby modulating cell function (11,12). Accordingly, it is conceivable that CL-11 can modulate DC function (in an autocrine or paracrine manner) through engagement of carbohydrate ligand on the cell surface. Apart from the extracellular effect, CL-11 may exert a suppressive effect on DCs through other mechanisms such as intracellular actions, which warrants further investigation. Therefore, ablation of CL-11 could result in loss of suppressive effects on DCs, leading to cellular overactivation.

Based on our findings and previously published observations, we propose that CL-11 is an important negative regulator of APC activation (Figure 6). CL-11 deficiency causes APC overactivation, leading to enhanced T cell responses (particularly Th1/Th17). This may (directly or indirectly) promote systemic and local inflammatory responses and antigen-specific antibody production, thus contributing to CIA. In contrast, with CL-11 sufficiency, CL-11 mediates suppression of APC activation and subsequent T cell responses, resulting in minimal inflammation and tissue damage.

Another important finding of this study is that early administration of rCL-11 reduces the severity of CIA in mice. Given that the amino acid sequence of CL-11 is highly conserved between human and mouse, the efficacy of rCL-11 in protection against CIA in mice may have therapeutic implications for human RA (e.g., RA at preclinical stage or in first-degree relatives with anti-CCP positivity). The mechanisms by which administration of rCL-11 mediates protection are unclear. There are several potential mechanisms. First, rCL-11 could modulate the activation/function of APCs (e.g., monocytes) both in the circulation and lymphoid tissues, as rCL-11 can penetrate lymphoid tissues (Supplementary Figure 4, http://onlinelibrary.wiley.com/doi/10.1002/art.41696/abstract). Second, rCL-11 may mediate direct effects on T cell activation. Third, rCL-11 may mediate antiinflammatory effects on innate immune cells. Administration of rCL-11 after the onset of disease also resulted in a certain degree of protection against CIA, which may reflect the involvement of a direct inhibitory effect of CL-11 on T cell activation and/or CL-11–mediated antiinflammatory effects on monocytes/macrophages.

Our findings in the preclinical CIA model of RA and the availability of an ELISA that is specific and sensitive for human
CL-11 (28) led us to examine the clinical relevance of CL-11 in RA patients. By assessing serum CL-11 levels in RA patients and healthy controls and by performing correlation analyses, we determined that serum CL-11 levels were inversely associated with the DAS28 and key inflammatory markers such as ESR and CRP level. The mechanisms involved in the presence of low levels of CL-11 in RA are unknown but could result from dysregulated synthesis of CL-11, hyper-consumption, or genetic variations that exist in the general population (29), which warrants further investigation. Our findings are consistent with previous observations that patients with systemic lupus erythematosus have significantly lower serum CL-11 levels compared to controls (30), suggesting an association between lower serum CL-11 levels and autoimmune disorders.

Considering the spectrum of biologic functions of collectins, studies in surfactant proteins and MBL have suggested that binding of collectins to carbohydrate ligands on the surface of pathogens or host cells not only leads to activation of the lectin pathway but also mediates regulation of multiple cellular processes (31,32). This also appears to be true for CL-11. Studies in patients with Malpuech-Michels-Mingarelli-Carnevale syndrome, murine models of renal ischemia reperfusion injury, and Streptococcus pneumoniae infection in the lung suggest that the role of CL-11 in these pathologies is dependent on complement activation. However, complement activation–independent functions of CL-11, such as opsonophagocytosis of apoptotic cells and cell proliferation, have also been reported in previous studies (11,12). Furthermore, in the present study, no significant differences in the circulating levels of C3a/C5a and synovial C3d deposition between WT mice with CIA and Colec11−/− mice with CIA were observed (Supplementary Figures 5 and 6, http://onlinelibrary.wiley.com/doi/10.1002/art.41696/abstract), suggesting that the protective role of CL-11 observed in this model was achieved independently of complement activation. Overall, these studies support the notion that CL-11 as a multifunctional molecule participates in pathophysiology via different mechanisms, in a complement activation–dependent or –independent manner.

There are limitations to this study. Being focused on the mechanism of CL-11–mediated suppression of APC activation/function does not exclude the possibility that CL-11 may also provide protection against CIA by influencing other immune cell functions (e.g., T cells, macrophages, and regulatory T cells). Additionally, our study does not address how ablation of CL-11 causes overactivation of APC, which warrants further studies. As a paradigm shift in immune response regulation may exist in CIA, proinflammatory cytokines, especially for IFNγ, may change to having antiinflammatory effects. Our study therefore does not exclude the possibility that up-regulation of IFNγ in Colec11−/− mice with CIA might also exert antiinflammatory effects in this model. Finally, the sample size for the RA patient study was too small, and therefore the results may not be completely generalizable.

In conclusion, our findings reveal a novel role of CL-11 in protection against CIA in mice, suggesting that CL-11 confers protection through suppression of both APC activation and subsequent Th1 and Th17 responses. In addition, our data show that CL-11 plays an important role in limiting the development of RA and should be explored as a possible future treatment option.

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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. Drs. Zhou and K. Li 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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