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Deficiency of cathepsin K prevents inflammation and bone erosion in rheumatoid arthritis and periodontitis and reveals its shared osteoimmune role

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ABSTRACT

Using rheumatoid arthritis (RA) and periodontitis mouse models, we demonstrate that RA and periodontitis share many pathological features, such as deregulated cytokine production, increased immune-cell infiltration, increased expression of Toll-like receptors (TLRs), and enhanced osteoclast activity and bone erosion. We reveal that genetic deletion of cathepsin K (Ctsk) caused a radical reduction in inflammation and bone erosion within RA joint capsules and periodontal lesions, a drastic decrease in immune-cell infiltration, and a significant reduction in osteoclasts, macrophages, dendritic and T-cells. Deficiency of Ctsk greatly decreased the expression of TLR-4, 5, and 9 and their downstream cytokines in periodontal gingival epithelial lesions and synovial RA lesions. Hence, Ctsk may be targeted to treat RA and periodontitis simultaneously due to its shared osteoimmune role.
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1. Introduction

Periodontitis is one of the most common oral inflammatory diseases. This disease may result in the destruction of periodontal tissues and alveolar bone, which ultimately leads to tooth loss [1,2]. Multiple bacterial infections are important factors for periodontitis [3]. During this process, different kinds of cells participate in the immune response, such as polymorphonuclear neutrophils (PMNs), macrophages, dendritic cells (DCs), and T cells [4]. The affected cells can induce the expression of many cytokines, including the receptor activator of nuclear factor kβ ligand (RANKL) which can promote osteoclast (OC) formation and the following periodontal bone resorption [5–7].

The development of rheumatoid arthritis (RA) is followed by the activation of the immune system, resulting in the infiltration of immune cells in the synovial membrane of the joint [8]. RA is considered to be a chronic, immune-regulated inflammatory disease, which will cause an excessive inflammatory response, cartilage and bone destruction, and eventually structural damage and loss of function. RA is associated with significant morbidity and an increased risk of mortality [9]. A variety of studies have demonstrated a close association between RA and periodontitis [10–12]. Periodontitis and rheumatoid arthritis are inflammatory diseases enhanced by dysregulation of the immune response in the progression of these diseases [11,13]. Clinical studies show that RA is more prevalent in individuals with periodontitis and vice versa in comparison to the general population [14,15]. One of the major obstacles to finding a cure for both RA and periodontitis is that the unknown factors that drive persistent immune cellular activation and inflammatory mediator synthesis remain elusive [4,16].

Toll-like receptors (TLRs), which are an important part of the innate and adaptive immune system, have been proposed as driving the inflammation in RA. In the development of periodontitis, TLRs have also been shown to have a critical role in the

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periodontitis lesion area [17]. Cathepsin K (Ctsk) is a peptidase C1 protein family member that is known to be a lysosomal cysteine protease [18]. The function of cathepsin K in periodontitis has not been characterized and the role of Ctsk in TLR signaling pathways in periodontitis is unknown. There is limited study on the role of Ctsk in TLR signaling pathways in RA. The common pathogenesis between RA and periodontitis mediated by Ctsk has not yet been evaluated.

Therefore, we sought to understand the underlying pathogenesis by which Ctsk regulates both RA and periodontitis. Ctsk knockout mice were used in this study to investigate the function of Ctsk in the development of RA and periodontitis. Our data reveal that RA and periodontitis have a common aberrant high TLRs signaling pathogenesis and suggest the critical osteoimmune function of Ctsk in rheumatoid arthritis and periodontitis.

2. Materials and methods

For complete Materials and Methods, please see Supplemental Materials and Methods.

2.1. Infection with bacterial strains

The bacteria used in this study were *Porphyromonas gingivalis W50* (ATCC: 53978), *Treponema denticola* (ATCC: 35404) and *Tannerella forsythia* (ATCC: 43037). These strains were grown under anaerobic conditions (80% N_2, 10% H_2, and 10% CO_2) at 37 °C in a Coy anaerobic chamber and were cultured [19,20]. An equal volume of sterile 2% (w/v) CMC was added, mixed thoroughly, and 100 μl (5×10^9 cells of *P. gingivalis* per ml, 5×10^9 cells of *T. denticola* per ml and *T. forsythia* per ml) was administered by oral and anal topical application according to a previously described protocol [21,22].

2.2. Animals

Seven to eight-week-old female wild-type (WT) C57BL/6J mice, purchased from the Jackson Laboratory were used for the periodontitis experiments. $Ctsk^{-/-}$ mice were previously generated in our lab with the C57BL/6J background for periodontitis experiments [23]. C57BL/6J background $Ctsk^{-/-}$ and WT mice were crossed with human TNF-transgenic mice (hTNF-tg) mice to generate hTNF-tg $Ctsk^{-/-}$ and hTNF-tg WT mice for RA experiments. For the periodontitis experiments, mice were divided into 4 groups: (1) wild-type (WT) normal group; (2) $Ctsk^{-/-}$ normal group; (3) WT disease group (bacterial infection); and (4) $Ctsk^{-/-}$ disease group (bacterial infection). For the RA experiments, mice were divided into 4 groups: (1) WT mice, (2) $Ctsk^{-/-}$ mice, (3) hTNF-tg mice, and (4) hTNF-tg $Ctsk^{-/-}$ mice. Experiments in the current study were performed in triplicate on three independent occasions, and $N = 5$ for each group, resulting in a total sample number of $N = 15$ for each group. This study was approved by the University of Alabama at Birmingham (UAB) Institutional Animal Care and Use Committee (IACUC). The animals were maintained at the UAB animal facility and were given distilled water and allowed to feed freely.

3. Results

3.1. Knockout of Ctsk resulted in bone protection and the decrease of TLRs expression in the periodontitis lesion area

To test the possible role during the progression of periodontitis lesions mediated by Ctsk, we used the periodontal disease mouse model established by our previous study [21]. Samples from WT and $Ctsk^{-/-}$ mice with or without infection were analyzed by methylene blue staining eight weeks after initial infection (Fig. 1A). Vertical and horizontal observation showed that there was no obvious bone resorption in the control groups without infection. Bone resorption was significant in the WT periodontitis group, but not in the $Ctsk^{-/-}$ periodontitis group (red arrows) (Fig. 1A). However, the hTNFtg mice did not show spontaneous periodontitis at 16 weeks (Fig. S1). H&E staining of the periodontal tissues showed that bone destruction, as well as monocyte infiltration (red arrows), increased in the WT periodontitis group (Fig. 1B). Quantitative analysis of the alveolar bone resorption area and the length of alveolar bone loss revealed that each measurement was significantly higher in the WT periodontitis group than in the $Ctsk^{-/-}$ periodontitis group (Fig. 1C and D). TLRs are important for the innate immune response in inflammatory diseases. In bacterial-mediated inflammation, TLR4 recognizes lipopolysaccharide (LPS), TLR5 recognizes flagellin, and TLR9 recognizes bacterial DNA and CpG oligodeoxynucleotide, which are critical antigens that cause an immune response [24]. In the periodontitis lesion area, the expression of TLR4, 5, and 9 at eight weeks decreased significantly in the $Ctsk^{-/-}$ periodontitis group compared to the WT periodontitis group (Fig. 1E–J).

3.2. Ctsk knockout provided bone protective effects as well as a decreased innate immune response in RA

In our current study, we used the human TNF-transgenic mouse model to confirm the results (Fig. 2). X-ray analysis also showed significant bone destruction (white arrow) in the knee joint and hind ankle joint in the hTNF-tg group compared to the hTNF-tg $Ctsk^{-/-}$ group at 24 weeks (Fig. 2A–D). SO staining also showed that the cartilage has been protected in the hTNF-tg $Ctsk^{-/-}$ group (Fig. 2E and F). We also confirmed the expression of Ctsk in WT, $Ctsk^{-/-}$, hTNF-Tg and $Ctsk^{-/-}$ hTNF-Tg groups at 24 weeks. (Fig. S2). Ctsk was successfully depleted in the Ctsk knockout group in the RA and periodontitis lesion areas. To explore whether these TLRs are involved in the development of RA in the same manner as in the periodontitis lesion areas, and to determine whether Ctsk has an effect on the immune response during the progression of RA, we performed immunohistochemistry (IHC) staining of the RA lesion areas of different groups at 24 weeks (Fig. 2G–L). A significant increase of TLR4-positive, TLR5-positive, and TLR9-positive cells was observed in the RA lesion area in hTNF-tg mice compared to $Ctsk^{-/-}$ hTNF-tg mice, $Ctsk^{-/-}$ mice, or WT mice (Fig. 2G–L).

3.3. Ctsk knockout reduced immune-related cells in RA and periodontitis lesion areas

DCs are antigen presenting cells which will differentiate after exposure to proinflammatory cytokines, immune complexes, or endogenous inflammatory factors that are recognized by TLR [25]. Our results showed that there was significantly higher expression of the DCs marker CD11c in the RA lesion area in hTNF-tg mice compared to hTNF-tg $Ctsk^{-/-}$ mice (Fig. 3A–C). Similarly, there was a significant increase of CD11c-positive DCs in the periodontitis lesion area in the WT periodontitis group compared to the $Ctsk^{-/-}$ periodontitis group (Fig. 3D–F). This indicates that knockout of *Ctsk* has an impact on antigen-presenting cells in the RA and periodontitis lesion areas. We further performed IHC analysis of the macrophage-specific marker F4/80 on mutant mouse hTNF-tg RA (Fig. 4A). Quantification results showed that the F4/80-positive cells were lower in the $Ctsk^{-/-}$ RA groups (Fig. 4B). The current results revealed that OCs were activated in the RA group (Fig. 4C). The OC numbers in the $Ctsk^{-/-}$ hTNF-tg groups were significantly lower than in the hTNF-tg groups (Fig. 4D). Since activation of OCs not only depends on osteoblasts, but also the immune system, this indicates that Ctsk has an important function in the immune system. Interestingly, the IHC stain

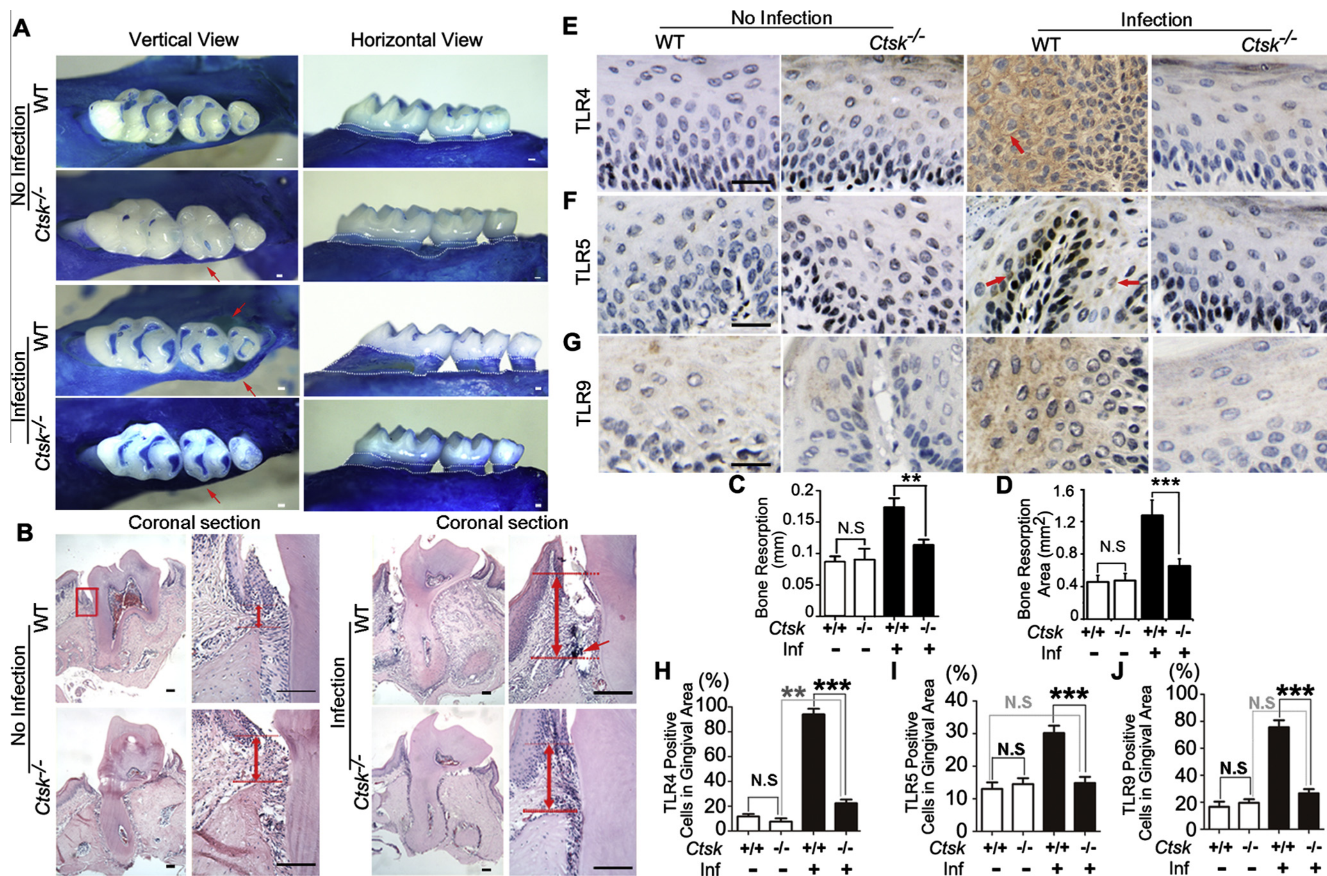

Fig. 1. Knockout of *Ctsk* resulted in bone protective effects in the periodontitis lesion area. (A) Methylene blue staining of the maxilla tooth from the WT and *Ctsk*$^{-/-}$ groups with and without infection at 8 weeks after initial infection. Red arrows indicate vertical bone resorption. White dot areas indicate horizontal bone resorption. (B) H&E stain of the periodontal tissue from the WT and *Ctsk*$^{-/-}$ groups with and without infection. Columns 2 are enlarged images of boxed areas in column 1. Red arrows indicate monocyte infiltration. Scale bar: 100 μm. (D) Quantification of horizontal alveolar bone resorption area in "A". (C) Quantification of alveolar bone resorption in "B". (E–J) IHC stains and quantification of TLR4-positive (E, H), TLR5-positive (F, I), and TLR9-positive (Brown) (G, J) cells in gingival areas in the WT and Ctsk$^{-/-}$ groups with and without infection at 8 weeks. Red arrows indicate positive cells. Inf: infection. **: $P < 0.01$, ***: $P < 0.001$. N.S: no significance. $N = 5$, repeated three times. Scale bar: 25 μm.

and Tartrate-resistant acid phosphatase (TRAP) stain similarly demonstrated that Ctsk has a role in immune cells as well as osteoclasts since F4/80-positive macrophages and TRAP-positive cells decreased significantly in the *Ctsk*$^{-/-}$ periodontitis group compared to the WT periodontitis group (Fig. 4E–H). In order for Ctsk to have an effect on the immune system, the immune cells must express Ctsk. Therefore we determined whether Ctsk was expressed in macrophages or DCs by co-localization analysis (Fig. 4J). The results showed that Ctsk was expressed in macrophages and DCs. To further confirm our findings, we applied CD3 immunofluorescence (IF) stain to examine T cell activation (Fig. 5). T lymphocytes are important immune effector cells that can be divided into cytotoxic T lymphocytes, helper T lymphocytes, and suppressor T lymphocytes. The co-surface marker of lymphocytes, CD3, can be used to detect activated T lymphocytes in inflammation [26]. The results showed that CD3-positive T cells decreased significantly in the lesion areas in the *Ctsk*$^{-/-}$ hTNF-tg group and *Ctsk*$^{-/-}$ periodontitis group (Fig. 5). These results indicated that *Ctsk* knockout may affect the immune response that occurs in both the RA and periodontitis lesion areas.

3.4. Inhibition of Ctsk reduced the expression of pro-inflammatory cytokines in the RA and periodontitis lesion areas

In the periodontitis lesion area at 8 weeks, the protein expression of TNF-α, IL-6 and IL-12 was significantly decreased in the *Ctsk*$^{-/-}$ periodontitis group when compared to the WT periodontitis group (Fig. 6A). qRT-PCR showed that the mRNA expression of TLR genes (*i.e. TLR4*, *TLR5* and *TLR9*) was significantly increased in the WT periodontitis group compared to the other groups (Fig. 6B). ELISA was used to evaluate the effect of Ctsk knockout on the protein expression levels of TNF-α, IL-1α, IL-6, IL-17, and IL-10, which are related to the TLR signaling pathway in inflammatory RA tissues. In the RA lesion area at 24 weeks, the protein expression of TNF-α, IL-6, IL-1α, and IL-17 was significantly increased in hTNF-tg mice compared to the normal control group (Fig. 6C). qRT-PCR revealed that the mRNA expression of *Ctsk*, pro-inflammatory genes (*i.e. IL-12b*) and TLR genes (*i.e. TLR4*, *TLR5* and *TLR9*) was significantly increased in the hTNF-tg group compared to the other groups (Fig. 6D). To confirm the possible role of Ctsk in the immune cell response to bacterial-induced inflammation through TLR, we used LPS and CpG to stimulate mouse bone marrow (MBM)-derived dendritic cells in vitro (Fig. 6E–G). Our in vitro data showed that *Ctsk* knockout greatly inhibited cytokine expression-related NF-κB activation. The expression of TNF-α, IL-6 and IL-12 in WT and *Ctsk*$^{-/-}$ DCs increased in CpG- and LPS-stimulated groups compared to unstimulated groups (Fig. 6E). However, the expression of inflammatory cytokines in the *Ctsk*$^{-/-}$ CpG stimulation group was significantly lower than in the WT CpG stimulation group. In the LPS stimulation group, although expression of inflammatory cytokines increased in WT and *Ctsk*$^{-/-}$ MBM DCs, the difference between the two groups was not statistically significant ($P > 0.05$). The mRNA expression of *IL-6* was consistent with the ELISA results, which was significantly lower in the *Ctsk*$^{-/-}$ CpG stimulation group than the WT CpG stimulation group. Although *IL-6* gene expression

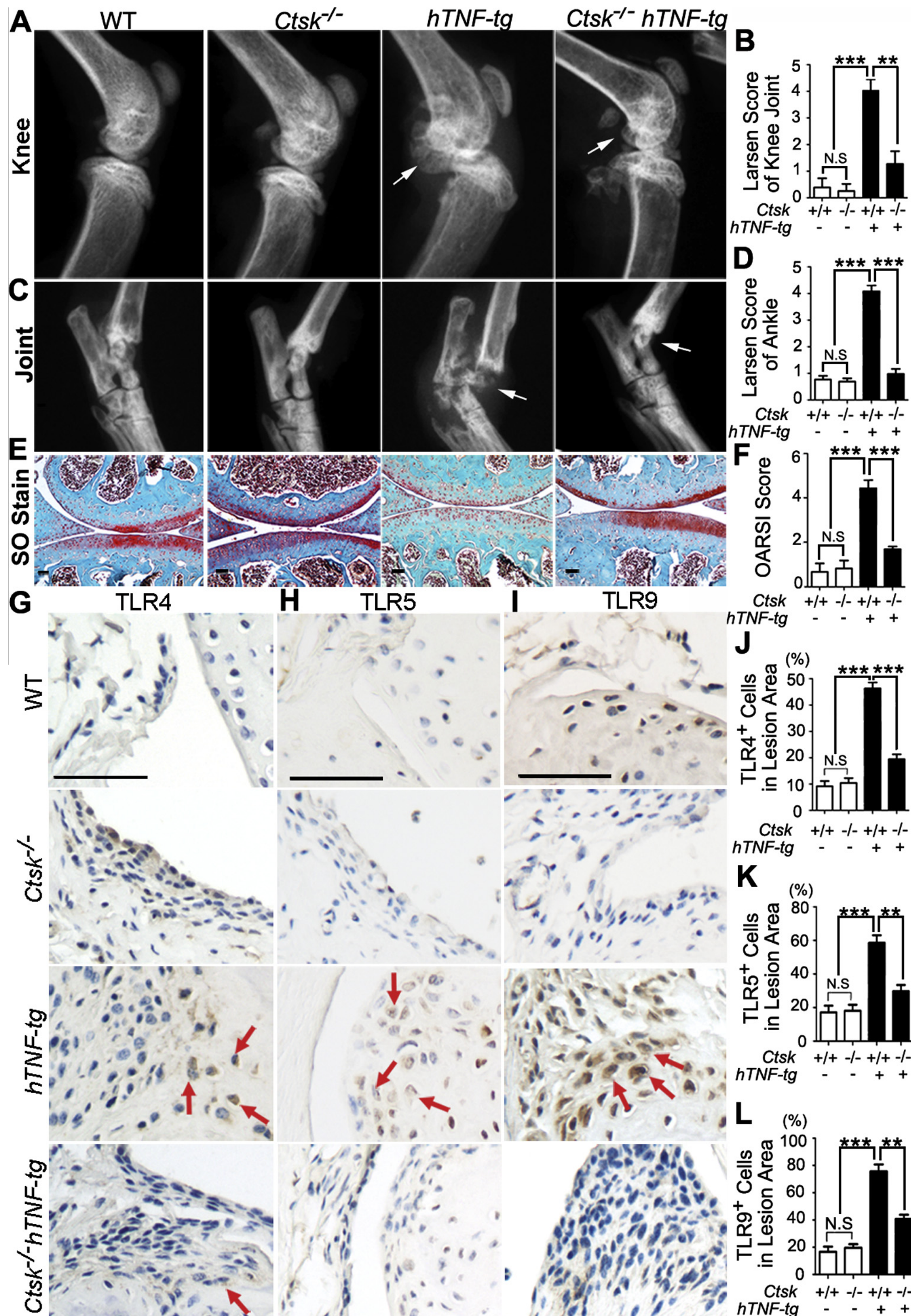

Fig. 2. *Ctsk* Knockout provided bone protection in rheumatoid arthritis (RA). (A–D) Radiographic and quantification analysis of joints from the WT, *Ctsk*$^{-/-}$, hTNF-Tg and *Ctsk*$^{-/-}$ hTNF-Tg groups at 24 weeks. X-ray analysis showed severe joint bone destruction (white arrows). (E, F) Safranin O stain and quantification analysis of joints from different groups. SO stain showed decreased cartilage in the hTNF-Tg group. Scale bar: 100 µm. (G–L) Immunohistochemical (IHC) stains and quantification of TLR4-positive cells (G, J), TLR5-positive cells (H, K), and TLR9-positive cells (I, L) in RA lesion areas in different groups at 24 weeks. **: $P < 0.01$, ***: $P < 0.001$. N.S: no significance. $N = 5$, repeated three times. Scale bar: 125 µm.

in the LPS-stimulated *Ctsk*$^{-/-}$ group was much higher than in the LPS-stimulated WT group, there was no significant difference in IL-6 protein expression between LPS-stimulated *Ctsk*$^{-/-}$ and WT groups. The expression of inflammatory factors (*i.e. IL-12b*) in the CpG-stimulated *Ctsk*$^{-/-}$ group was lower than the CpG-stimulated WT group, while expression of these inflammatory cytokines showed an opposite trend in the LPS-stimulated groups (Fig. 6F). Although Ctsk knockout has a significant impact on TLR9-mediated expression of inflammatory cytokines, its specific role in the signal transduction pathway is still unknown. Therefore, we tested the mRNA expression of M*yD88*, *TRAF6*, *IRAK1* and *IRAK4*, which are important in signal transduction pathways associated with TLR9. Results showed that Ctsk knockout influenced the CpG-stimulated expression of *MyD88*, *TRAF6*, *IRAK1* and *IRAK4* in the TLR9 signaling pathway (Fig. 6G). In the case of LPS stimulation, *IRAK1* decreased in the *Ctsk*$^{-/-}$ group, the expression of *MyD88* and *IRAK4* didn't

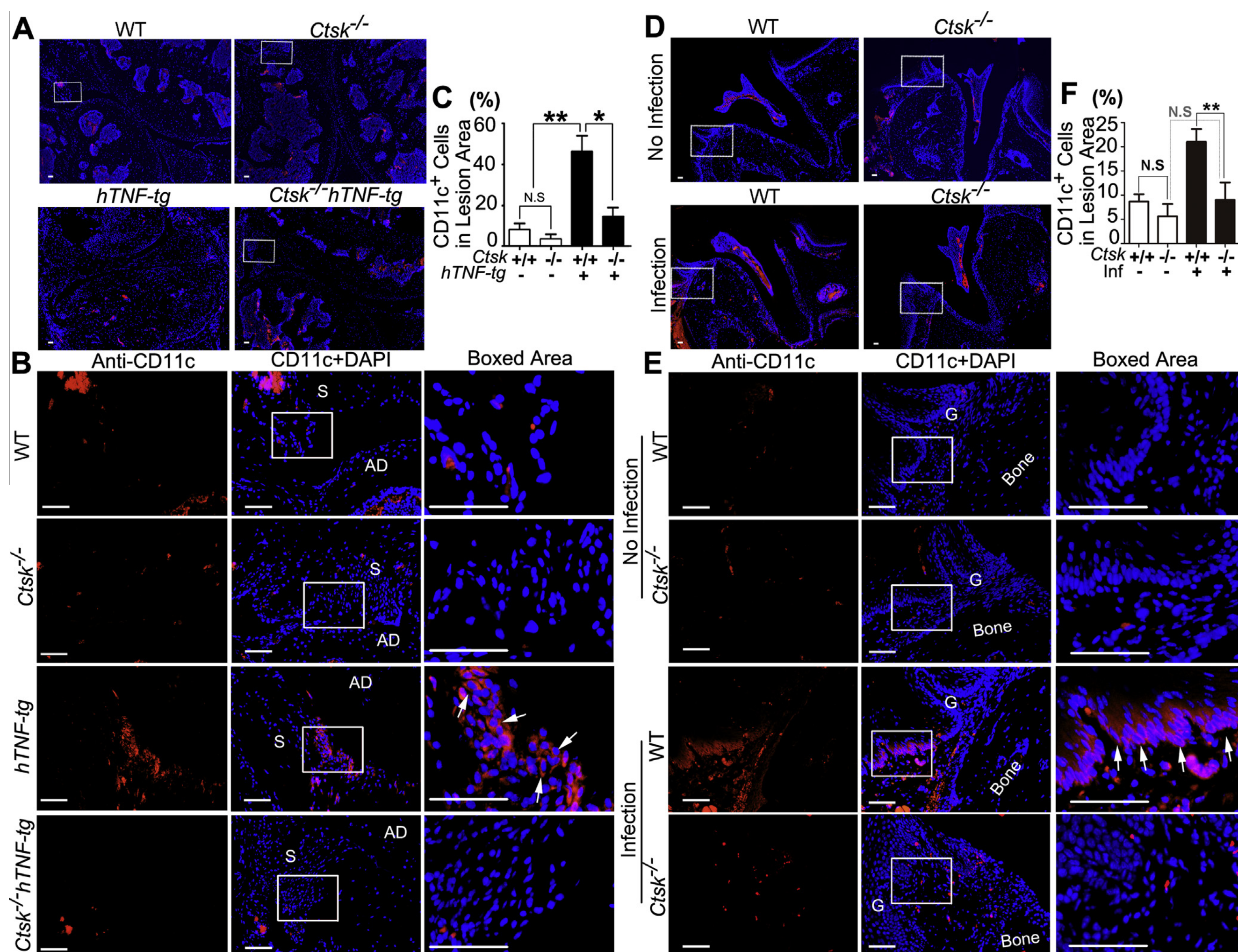

Fig. 3. Knockout of *Ctsk* reduced CD11c-positive dendritic cells in RA and periodontitis lesion areas. (A–C) Immunofluorescence (IF) staining and quantification of CD11c-positive (Texas red) dendritic cells (DCs) in the RA lesion area in different groups at 24 weeks. Boxed areas in "A" are magnified in columns 1 and 2 of "B". Boxed areas in "B" are magnified in column 3 of "B". White arrows indicate positive cells. (D–F) IF staining and quantification of CD11c-positive (Texas red) DCs in the periodontitis lesion area in different groups at 8 weeks. Boxed areas in "D" are magnified in columns 1 and 2 of "E". Boxed areas in "E" are magnified in column 3 of "E". White arrows indicate positive cells. S: synovial, AD: articular disc, G: gingival, Inf: infection. *: $P < 0.05$, **: $P < 0.01$. N.S: no significance. $N = 5$, repeated three times. Scale bar: 50 μm.

change in the *Ctsk*$^{-/-}$ and WT groups, and *TRAF6* levels increased in the *Ctsk*$^{-/-}$ group compared to the WT group (Fig. 6G).

4. Discussion

A previous study showed that knockout of Ctsk may partially inhibit bone destruction in hTNFtg mice [27]. Our current study suggests that the pathogenesis by which Ctsk mediates the immune response in RA and periodontitis is similar in the two diseases. Retrospective studies in recent years show that there is an interaction between periodontal disease and rheumatoid arthritis, compared with healthy people. The incidence rate of periodontal disease is higher in patients with rheumatoid arthritis, and in periodontal disease patients the incidence of rheumatoid arthritis is higher [28]. Recent studies have established the link between RA and periodontitis, claiming that periodontitis might be a causal factor that will enhance the severity of RA and vice versa, and these two diseases are highly associated at the epidemiological level [10,14]. Synovial tissue in the RA lesion area is considered to be the inflammation center and a source of osteoclast differentiation for the development of RA [29–31], while periodontitis is always preceded by inflammation of gingival tissue (gingivitis) [32]. Our present findings demonstrate that DCs, macrophages, and T cells are infiltrated extensively in the RA synovial tissue and periodontitis gingival tissue. The synovial fibroblasts and gingival epithelial cells may have similar functions in RA and periodontitis: initiating the innate response, activating immature DCs to mature DCs, and mediating inflammation cytokine expression and OC activation which further tissue damage. The inhibition of Ctsk results in the suppression of immune cell expression, osteoclast activation, and the prevention of articular cartilage erosion and alveolar bone loss in the hTNF-tg mouse models and periodontitis mouse model respectively. Cathepsin K was first discovered and cloned by our lab [33,34], which was reported to play important function in immune cells [35]. Additional studies are needed to distinguish between these possibilities [36]. Previous studies have shown that Ctsk knockout has no effect on OC differentiation [23]. However, we found a significant decrease in OCs in the *Ctsk*$^{-/-}$ RA and *Ctsk*$^{-/-}$ periodontitis groups, indicating Ctsk's role in inflammation. Our current results suggest that Ctsk may mediate RA and periodontitis bone destruction not only through its known function in OCs, but also through immune cell-mediated activation of OCs.

Fig. 4. Knockout of *Ctsk* reduced macrophages and osteoclasts in RA and periodontitis lesion areas. (A, B) IHC stains and quantification of F4/80 positive (Brown) cells in RA lesion areas in different groups at 24 weeks. Second column of "A" are enlarged images of red boxed areas. Red arrows indicate positive cells. (C, D) TRAP stain and quantification of osteoclasts in RA lesion areas in different groups at 24 weeks. (E, F) IHC stains and quantification of F4/80 positive (Brown) macrophages in the periodontitis lesion areas of the WT and *Ctsk*$^{-/-}$ groups with and without infection at 8 weeks. Red arrow and white inset box of enlarged image show positive cells. (G, H) TRAP stain and quantification of osteoclasts in the periodontitis lesion areas in different groups at 8 weeks. (I) Normal serum at the same area served as a negative control. (J) Ctsk localizes to DCs and macrophages in the periodontitis lesion area. White arrows and white inset boxes of enlarged images show double positive cells. Inf: infection, DIC: differential interference contrast. **: $P < 0.01$, ***: $P < 0.001$. N.S: no significance. $N = 5$, repeated three times. Scale bar: 100 μm.

Asagiri et al. further demonstrated that knockout of *Ctsk* can reduce the inflammation of RA in a rat model [37]. Macrophages and DCs are critically involved in the pathogenesis of RA as shown in our current studies. Not only do they produce a variety of pro-inflammatory cytokines and chemokines, but they also contribute to the cartilage and bone destruction in RA [38]. Our current study showed that Ctsk may play a critical role in the immune response of RA and in the periodontitis lesion area. IF was used in our study to detect the expression of Ctsk in immune cells, which would be a prerequisite for Ctsk having any function in the immune system.

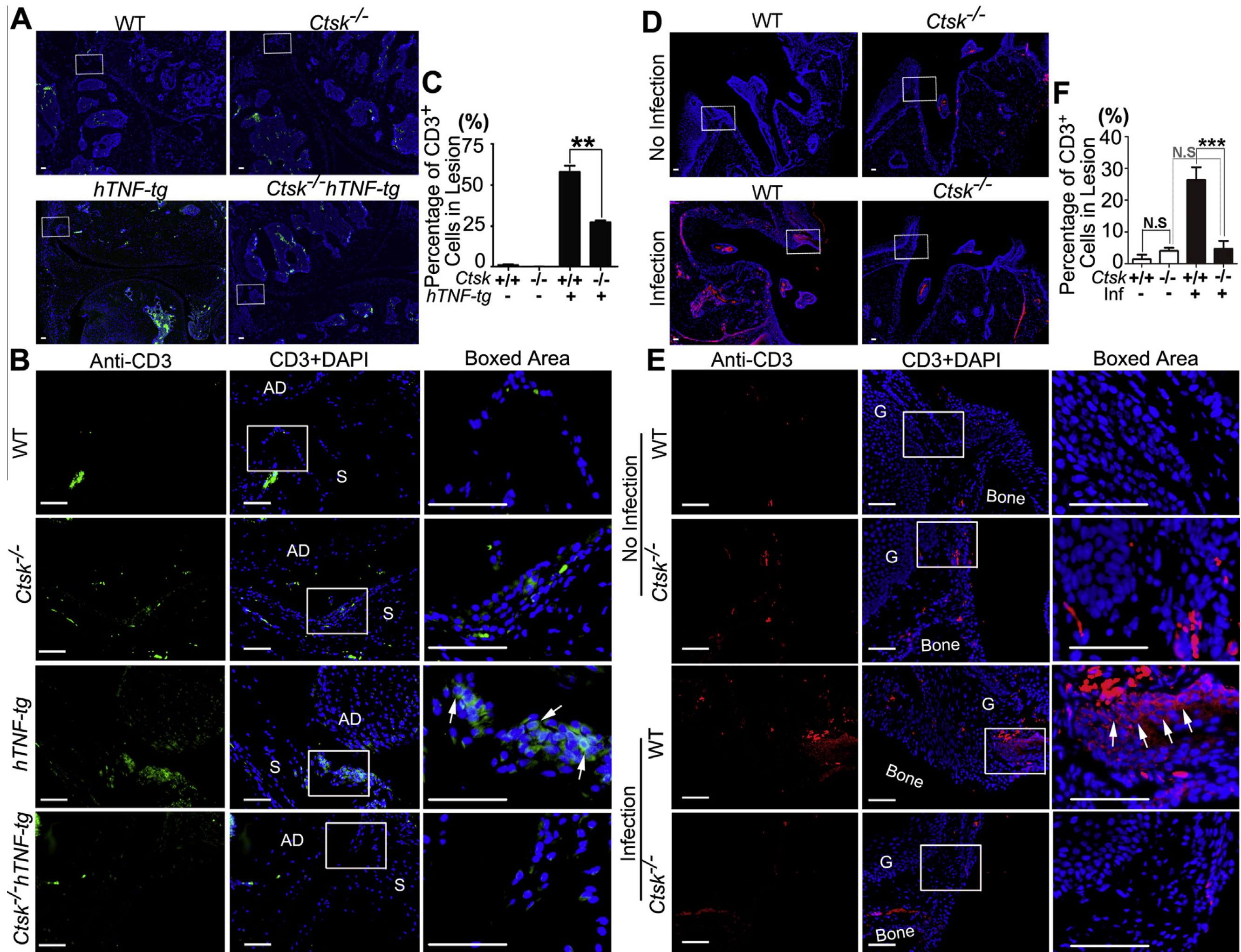

Fig. 5. Knockout of *Ctsk* reduced CD3-positive T cells in RA and periodontitis lesion areas. (A–C) IF staining and quantification of CD3-positive (GFP) T cells in the RA lesion area in different groups at 24 weeks. Boxed areas in "A" are magnified in columns 1 and 2 of "B". Boxed areas in "B" are magnified in column 3 of "B". White arrows indicate positive cells. (D–F) IF staining and quantification of CD3-positive (Texas red) T cells in the periodontitis lesion area in different groups at 8 weeks. Boxed areas in "D" are magnified in columns 1 and 2 of "E". Boxed areas in "E" are magnified in column 3 of "E". White arrows indicate positive cells. S: synovial, AD: articular disc, G: gingival, Inf: infection. **: $P < 0.01$, ***: $P < 0.001$. N.S: no significance. $N = 5$, repeated three times. Scale bar: 50 μm.

Our findings showed that Ctsk is expressed in immune cells, such as macrophages and DCs. We have shown that knocking out *Ctsk* has reduced the pro-inflammatory cytokine expression in RA and periodontitis lesion tissues. Many of these effector molecules appear to be common to both diseases. While the effects of cytokines on normal cellular processes are important, it is their purported roles in the immune response, which may involve excessive production, dysregulation, or inadequate inhibition, that may be critical for the progression of both RA and periodontitis disease. Periodontitis has very similar cytokine profiles to RA based on our current study, which consists of persistently high levels of pro-inflammatory cytokines (*e.g.* TNF-α). In a rheumatoid arthritis rat model, Asagiri et al. verified that Ctsk possibly mediates the immune response of DCs in vitro [37]. These results showed that, as a dual role protein, Ctsk not only participated in osteoclastic bone resorption, but was also involved in immune cell-mediated inflammation.

Importantly, the present study provides new insights into the possible connections of the immune response to RA and periodontitis. In the periodontitis mouse model, knockout of Ctsk resulted in similar decreases in TLR4, 5 and 9. Although the RA models we used do not involve bacteria-induced inflammation lesions, our data suggested that TLR4, 5 and 9 also decreased significantly in the hTNF-tg *Ctsk*$^{-/-}$ group. This indicates that TLR4, 5 and 9 may play similar functions of mediating the immune response in RA and periodontitis lesion areas and that Ctsk may regulate this process. ELISA and qRT-PCR results confirmed the in vivo IHC stain in both RA knee tissues and periodontitis lesion tissues. These results underscore the possibility that future therapies could block Ctsk in RA and periodontitis by specific small molecule inhibitors or gene therapy. However, the pathogenesis by which Ctsk functions in TLRs activation is still unclear. The current view is that cathepsins may make TLRs form a correct protein structure through proteolytic processing, thereby initiating the antigen recognition process [39,40]. MyD88-related transcription level, which is an important component of the activation of the downstream pathway, was significantly inhibited in *Ctsk*$^{-/-}$ DCs with CpG stimulation. However, MyD88, TRAF6, IRAK1, and IRAK4 transcription levels were not fully inhibited in LPS-stimulated *Ctsk*$^{-/-}$ DCs.

In conclusion, our study first demonstrated that bone resorption and exaggerated inflammatory host responses in periodontitis and RA are mediated by Ctsk through the TLR4, 5 and 9 signaling pathways in DCs. These observations highlight a central pathogenic role for Ctsk in these settings and identify Ctsk as a directed, logical

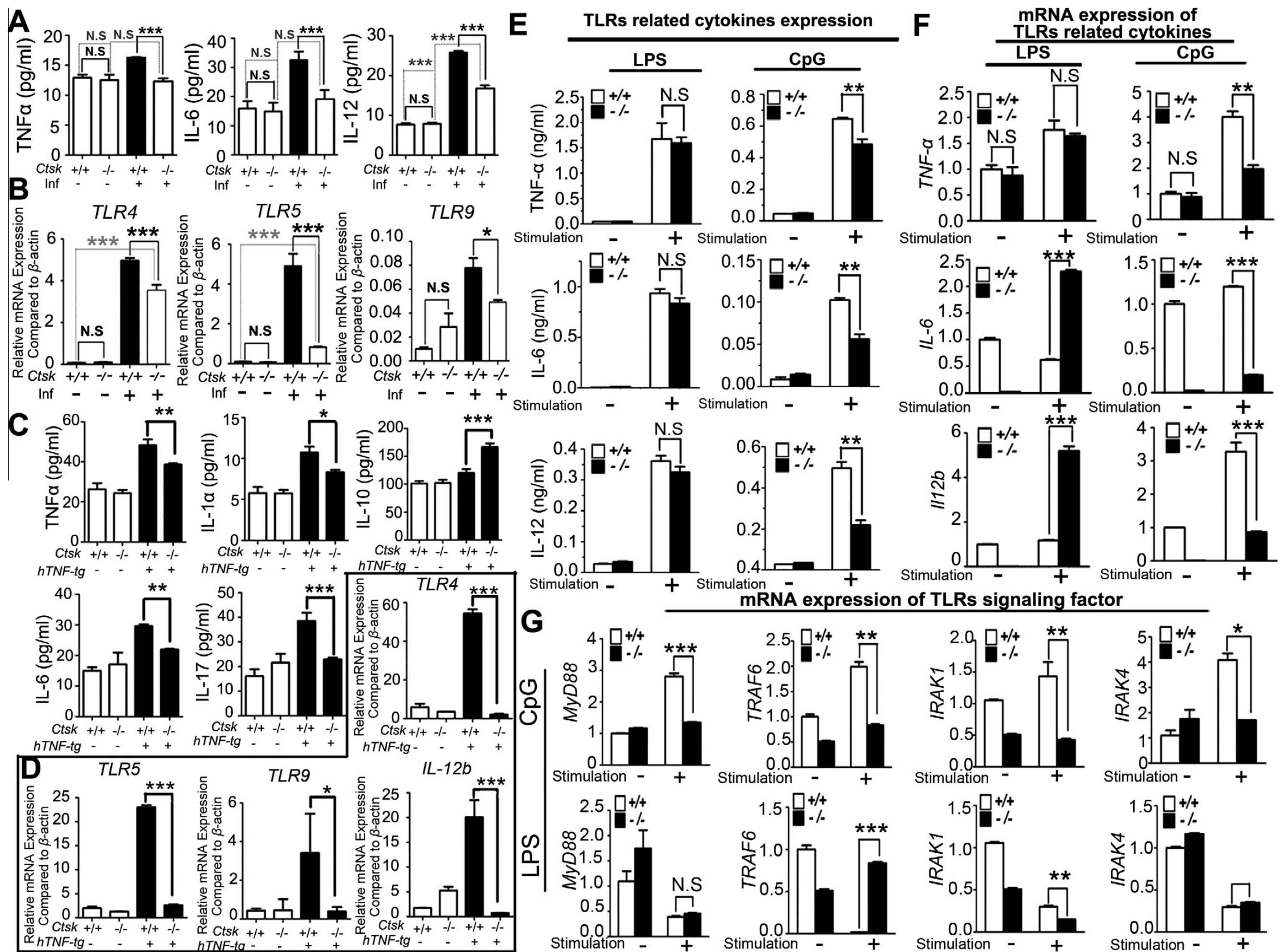

Fig. 6. Ctsk knockout or inhibition reduced the expression of pro-inflammatory cytokines in vivo (RA and periodontitis lesion areas) and in vitro (WT and *Ctsk*$^{-/-}$ DCs). (A) Cytokine expression levels of TNF-α, IL-6 and IL-12 in the periodontitis lesion area were detected by ELISA at 8 weeks. (B) qRT-PCR of TLR genes in different groups. *β-actin* was used as an endogenous control. C, Cytokine expression levels of TNF-α, IL-1α, IL-6, IL-17 and IL-10 in the RA lesion at 24 weeks were detected by ELISA. (D) qRT-PCR of pro-inflammatory genes (*i.e. IL-12b*) and TLR genes in the RA lesion area at 24 weeks. (E) MBM-derived DCs from WT and *Ctsk*$^{-/-}$ mice were stimulated with LPS (200 ng/ml) or CpG (1 μM/ml) for 12 h. The production of TNF-α, IL-6, and IL-12 was measured by ELISA. (F) qRT-PCR analysis of *TNFα*, *IL-6*, and *IL-12b* expression in WT and *Ctsk*$^{-/-}$ MBM-derived DCs stimulated with LPS or CpG as above. (G) MBM-derived DCs from WT and *Ctsk*$^{-/-}$ mice were stimulated with LPS or CpG for 12 h. The expressions of TLRs downstream signaling pathway were detected by qRT-PCR. Inf: infection. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$. N.S: no significance. $N = 5$, repeated three times.

target for the suppression of cartilage and periodontal bone destruction. Indeed, optimal control of RA or periodontitis inflammation is most likely to preserve a range of homeostatic properties for both diseases. Collectively, Ctsk may be one possible target to treat RA and periodontitis simultaneously due to its shared pathogenesis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2015.04.008.

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