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Critical roles for CCR2 and the therapeutic potential of cenicriviroc in periodontitis: A pre-clinical study

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Abstract

Aim: CCR2 plays important roles in many inflammatory and bone metabolic diseases, but its specific role in periodontitis is unknown. In the present study, we aimed to explore the role of CCR2 in the progression of periodontitis and evaluate the effect of cenicriviroc (CVC) on periodontitis.

Materials and Methods: The expression of CCR2 was studied in patients with periodontitis and in ligation-induced murine model of periodontitis. The role of CCR2 in promoting inflammation and bone resorption in periodontitis was evaluated in *Ccr2*$^{-/-}$ mice and wild-type mice. The effect of CVC in the prevention and treatment of periodontitis was evaluated by systemic and local medication. Microcomputed tomography, haematoxylin and eosin staining, tartrate-resistant acid phosphatase staining, quantitative real-time polymerase chain reaction, enzyme-linked immunosorbent assay, and flow cytometry were used for histomorphology, molecular biology, and cytology analysis, respectively.

Results: In this study, we demonstrated that CCR2 was highly expressed in human and murine periodontitis and that CCR2 deficiency was associated with decreased inflammatory monocyte and macrophage infiltration and inflammatory mediators, osteoclast number and alveolar bone resorption. Prevention and treatment with CVC

Wenting Jiang and Tao Xu contributed equally to this work.

significantly reduced the severity of periodontitis, regardless of whether it was administered systemically or locally.

Conclusions: CCR2 plays an important role in the development and progression of periodontitis, and CVC is a potential drug for the prevention and treatment of periodontitis.

KEYWORDS
CCR2, cenicriviroc, monocytes/macrophages, periodontitis, targeted therapy

Clinical Relevance

Scientific rationale for study: CCR2 may promote the development of periodontitis by promoting the recruitment of monocytes/macrophages, but its specific role in the pathogenesis of periodontitis remains unclear. The effect of cenicriviroc (CVC) on periodontitis has not been reported.

Principal findings: We demonstrated for the first time that CCR2 was positively correlated with probing depths and that CCR2 deficiency was associated with lower periodontitis severity. Targeted CCR2/5 by CVC can inhibit the development of periodontitis.

Practical implications: Our study provides a new therapeutic target regarding host immunoregulation for periodontitis, and CVC may be a potentially effective drug for periodontitis prevention and treatment.

1 | INTRODUCTION

Periodontitis is a multifactorial inflammatory disease (Slots, 2017). The main pathological changes of periodontitis include substantial inflammatory cell infiltration, an increased number of osteoclasts, and degradation of soft and hard tissues (Kinane et al., 2017). Although dental plaque biofilm is the initial factor, the major tissue damage caused by periodontitis is attributed to the host immune response to microbial challenges (Hajishengallis et al., 2020). However, the pathogenesis of periodontitis involving host factors is complicated and not completely understood.

Monocytes/macrophages are important participants in the inflammatory response and bone resorption of periodontitis (Almubarak et al., 2020; Yao et al., 2021). The recruitment of monocytes/macrophages depends on chemokine signals. CCL2-CCR2 signalling-mediated migration of monocytes and monocyte-derived macrophages is involved in several inflammatory diseases and bone metabolic diseases. In osteoarthritis, knocking out CCR2 reduced inflammation and cartilage destruction by decreasing monocyte/macrophage numbers in mouse joints (Raghu et al., 2017). In osteoporosis, CCR2 knockout mice had a higher bone mass than wild-type (WT) mice due to a decrease in the number, size, and function of osteoclasts (Binder et al., 2009).

In the periodontal field, existing studies on CCL2-CCR2 signalling mainly focused on the analysis of clinical samples. Compared with healthy individuals, patients with periodontitis have higher expression levels of CCL2 in gingival crevicular fluid, saliva, and plasma, while after treatment, CCL2 expression is significantly down-regulated (Pradeep et al., 2009; Gupta et al., 2013; Boström et al., 2015; Gunpinar et al., 2017). However, the effect of CCR2 on periodontal inflammation and alveolar bone resorption caused by periodontitis has not been reported.

Initial periodontal therapy can effectively eliminate pathogenic plaque biofilm. However, not all patients responded well to this kind of therapy, it requires life-long maintenance, and the recurrence rate is high (Preshaw, 2018), indicating that an alternative therapeutic approach is urgently needed. Cenicriviroc (CVC) is a new dual antagonist that can inhibit both the CCR2 and CCR5 receptors (Lalezari et al., 2011), which can effectively ameliorate steatohepatitis and liver fibrosis by inhibiting the infiltration of monocyte-derived macrophages (Krenkel et al., 2018; Ambade et al., 2019). Long-term clinical trials have shown its favourable safety (Thompson et al., 2016; Friedman et al., 2018; Anstee et al., 2020).

The aim of the present study was to thoroughly explore the role of CCR2 in the progression of periodontitis and to evaluate the effects of systemically or locally administered CVC on periodontal inflammation and alveolar bone resorption in experimental periodontitis.

2 | MATERIALS AND METHODS

2.1 | Human samples

Twelve periodontally healthy individuals and 33 patients with periodontitis were recruited from the Department of Periodontology, Peking University Hospital of Stomatology, China. The inclusion criteria were described in our previous study (Shi et al., 2018). Briefly, healthy participants had no teeth with probing depth >3 mm, no sites with attachment loss, and no history of periodontitis; periodontitis

patients were included based on the presence of at least eight teeth with probing depth ≥4 mm and radiographic bone loss. Healthy gingival tissue samples were collected from subjects who received crown-lengthening surgery. Periodontitis-affected tissues were from sites with severe periodontitis and received tooth extraction and alveolar ridge preservation (Huang et al., 2019). The harvested tissues were stored at −80°C for total RNA and protein extraction.

The present study was approved by the Ethics Committee of the Peking University Health Science Center (PKUSSIRB-201310068a). All enrolled individuals provided written informed consent. The demographic and clinical characteristics of the study population are shown in Supplementary Tables 1 and 2.

2.2 | Mice

Specific pathogen-free C57BL/6 mice were purchased from SPF Biotechnology (SPF [Beijing] Biotechnology Co., Ltd., Beijing, China). CCR2 knockout (*Ccr2*$^{-/-}$) mice on a C57BL/6 background were obtained from Professor Yu Zhang (Department of Immunology, School of Basic Medical Sciences, Peking University). Littermate mice were produced from *Ccr2*$^{+/-}$ heterozygous mice. All experiments used 8-week-old male mice. All animals were maintained in specific pathogen-free conditions. All experiments were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals and with the approval of the Ethics Committee of Peking University Health Science Center (LA2021494). All data derived from animal studies were analysed by an experimenter blind to experimental conditions. The sample size of each experiment was determined based on the effect size, assumed significance level (.05), and assumed power (90%) (Festing & Altman, 2002), and the effect size was obtained according to standard deviation and mean difference of the pilot studies. The specific sample size of each experiment was described in the figure legends, and a total of 276 mice were used in the present study.

2.3 | Animal experiments

Experimental periodontitis in mice was established as previously described (Abe & Hajishengallis, 2013). Briefly, the maxillary second molars of mice were ligated with a 5–0 silk ligature for 7 days. Control mice underwent the same procedure but without ligation. To determine the preventive and therapeutic effects of CVC on periodontitis, CVC was administered systemically and locally. For systemic treatment, mice with ligature-induced periodontitis were given CVC (15 mg/kg body weight) or an equal amount of vehicle daily by subcutaneous injection from the day of ligation (Day 0), referring to the previous study (Ambade et al., 2019). For local treatment, to avoid mechanical damage to gums induced by injection, we injected CVC (1.5 mg/kg body weight) or vehicle on the ligation around the maxillary second molar using a graded Hamilton syringe (33-G needle) daily, and the single dose of CVC was determined according to results of the pilot study (Figure S1). For the preventive group, animals received medical intervention from Day 0 until they were sacrificed on Day 7. For the therapeutic group, animals received the same dose of drugs daily from Day 3 to Day 7 after ligature placement. Mice were randomly divided into experimental groups and control groups in each experiment.

For further details of the materials and methods used in the present study, please refer to Supplementary Information.

3 | RESULTS

3.1 | CCR2 and CCL2 are up-regulated in human and murine periodontitis

To evaluate the expression of CCR2 and CCL2 in periodontal tissues, we collected gingival tissues excised during clinical surgeries for detection. The transcriptional levels of CCR2 and CCL2 (Figure 1a) and the protein level of CCL2 were significantly up-regulated in periodontitis-affected tissues after adjusting for age and gender (Figure 1b). Moreover, these increases were positively associated with the severity of periodontitis (Figure 1c,d), which was indicated by using probing depths. quantitative real-time polymerase chain reaction (Real-time qPCR) analysis showed that proinflammatory cytokines tumour necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, and IL-17A were highly expressed in gingival tissues of the periodontitis group (Figure 1e). Further, correlation analysis revealed that there was a positive linear correlation between TNF-α, IL-1β, and IL-6 and probing depths, but there was no linear correlation between IL-17A and probing depths (Figure 1f). Moreover, CCL2, TNF-α, IL-1β, and IL-6, but not IL-17A, were positively correlated with CCR2 (Figure 1g).

To determine whether CCR2 and CCL2 were up-regulated during the progression of mouse periodontitis, we established mice models at different time points (Figure S2a-d). In accordance with the human data, the mRNA expression of CCR2 and CCL2 was also substantially higher in the mice with ligature-induced periodontitis than in the control mice, and significant differences began to appear on Day 3 after ligation (Figure S2e). Flow cytometry confirmed that the percentage of CD11b^{+}CCR2^{+} cells in the gingival tissue of the periodontitis group was increased significantly, with an increase of 4.2-fold on average (Figure S2f). Enzyme-linked immunosorbent assay confirmed the higher CCL2 protein levels in gingival periodontitis (Figure S2g).

3.2 | CCR2 deficiency protects against periodontitis in mice

To evaluate the role of CCR2 in periodontitis, we generated *Ccr2*$^{-/-}$ and WT littermate mice. A 7-day ligature-induced mouse model was established to evaluate the effect of CCR2 deficiency on periodontitis-induced inflammation and alveolar bone resorption (Figure 2a). No transcription of CCR2 was detected in the gingiva of the *Ccr2*$^{-/-}$ mice, indicating successful knockout (Figure 2b).

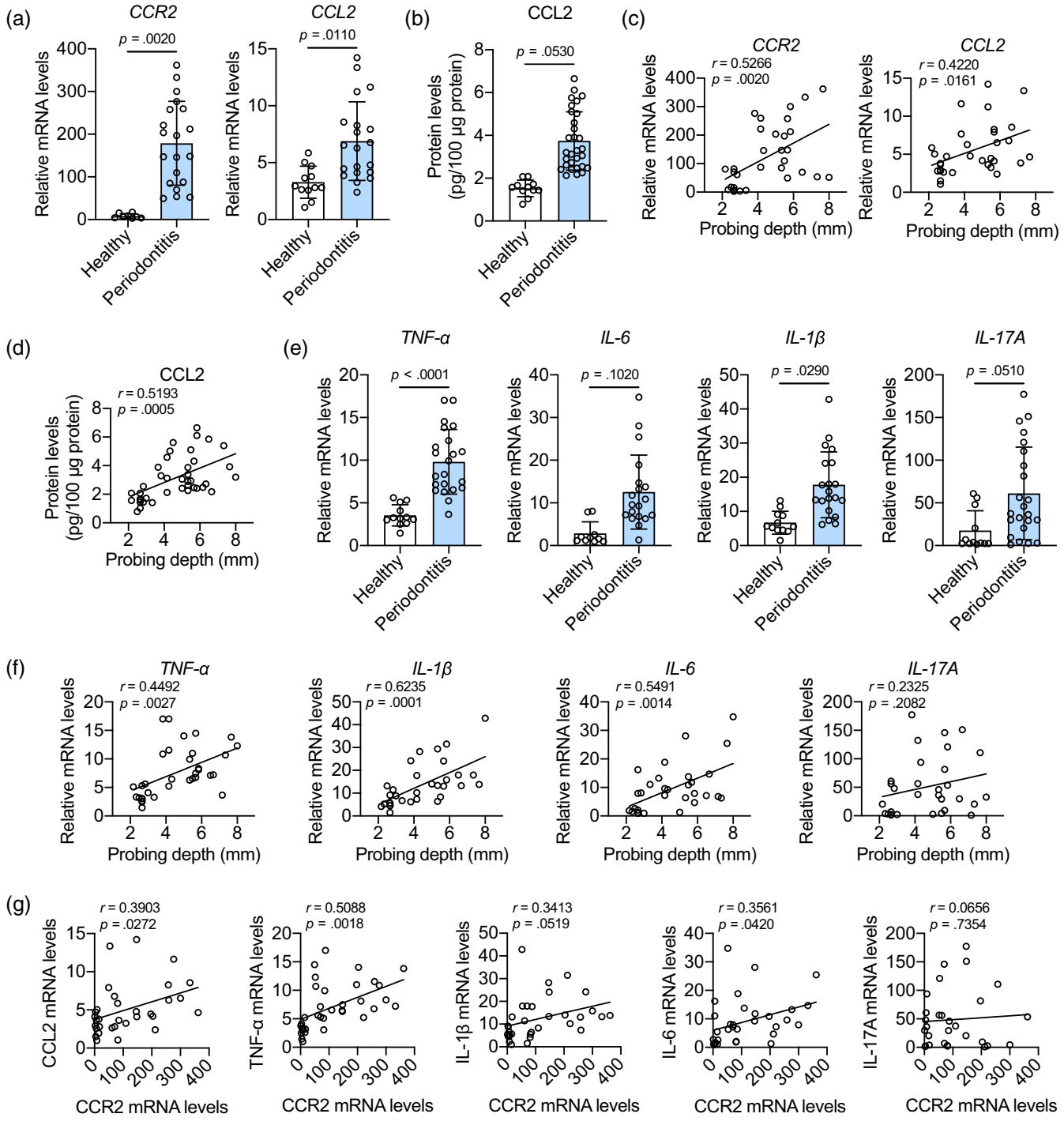

FIGURE 1 CCL2 and CCR2 levels are up-regulated in human periodontitis. (a) mRNA levels of CCR2 and CCL2 in gingival tissues of humans (healthy, $n = 11$; periodontitis, $n = 33$). (b) Protein level of CCL2 in gingival tissues of humans (healthy, $n = 12$; periodontitis, $n = 23$). (c) Correlation analysis of the mRNA levels of CCR2 and CCL2 and clinical probing depth. (d) Correlation analysis of the protein level of CCL2 and clinical probing depth. (e) mRNA levels of the proinflammatory cytokines tumour necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, and IL-17A in human gingival tissues. (f) Correlation analysis of mRNA levels of the proinflammatory cytokines TNF-α, IL-1β, IL-6, and IL-17A and clinical probing depth. (g) Correlation analysis of mRNA levels of the proinflammatory cytokines CCL2, TNF-α, IL-1β, IL-6, and IL-17A and the expression of CCR2. Data are representative of two independent experiments. Data are presented as the mean ± SEM. Statistical significance was determined by unpaired, two-tailed Student's *t* tests analysis of covariance, and Pearson correlation analysis

Morphologically, we intuitively found that the gingival swelling of the *Ccr2*$^{-/-}$ mice was significantly milder than that of the WT mice, in which the swollen gums almost covered the occlusal surface of the tooth (Figure 2c). Microcomputed tomography (micro-CT) scanning demonstrated that alveolar bone resorption was obviously reduced in the *Ccr2*$^{-/-}$ mice, and the percentage of residual bone volume in the

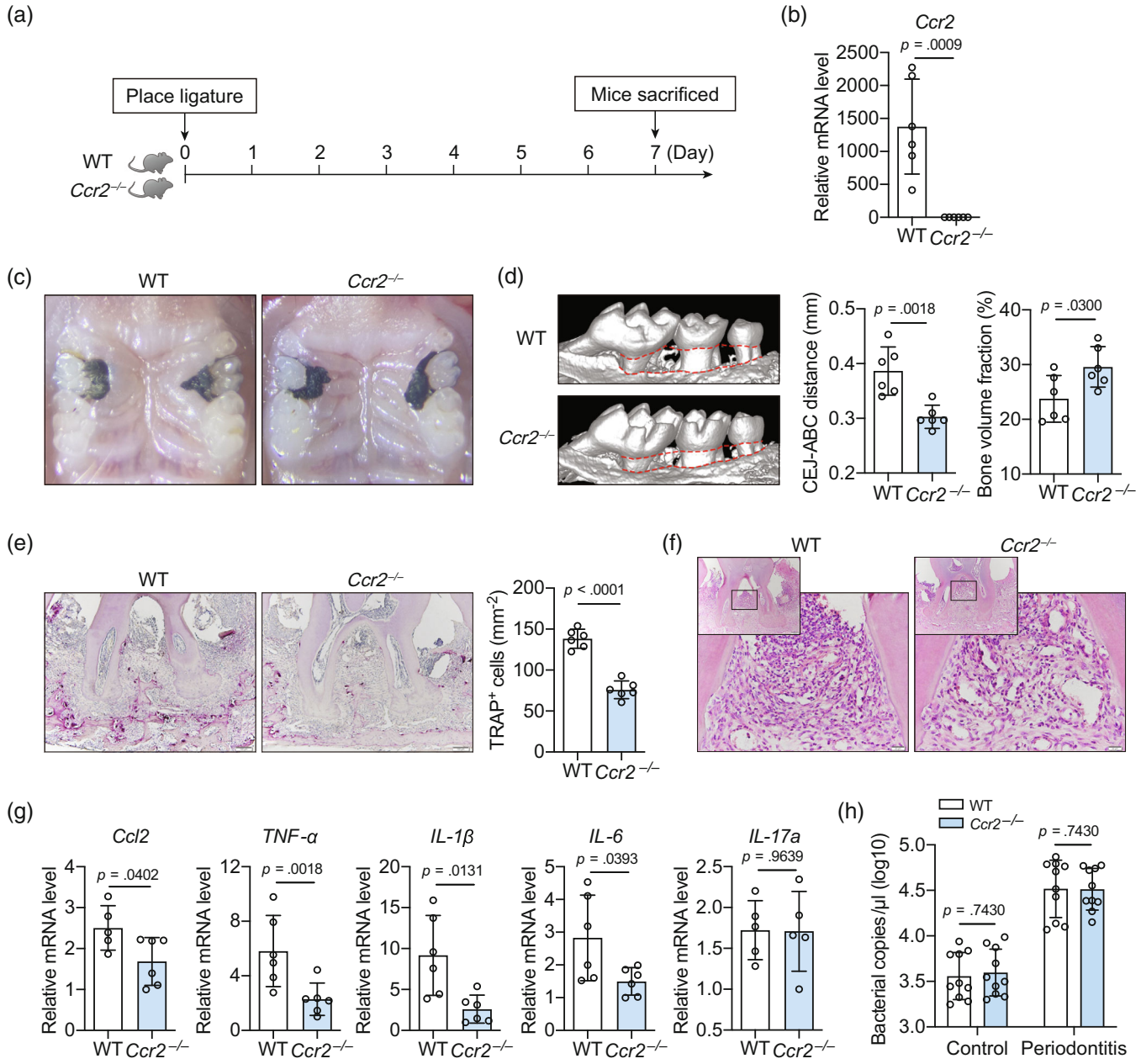

FIGURE 2 CCR2 deficiency inhibits periodontal inflammation and alveolar bone loss in mice with periodontitis. (a) Schematic diagram of the experimental design. (b) mRNA levels of CCR2 in murine gingival samples ($n = 6$). (c) Intra-oral images of mice were taken with a stereomicroscope. (d) Bone loss and bone volume fraction were analysed by micro-CT ($n = 6$). (e) The number of osteoclasts per square millimetre was analysed by tartrate-resistant acid phosphatase (TRAP) staining (scale bar = 100 μm, $n = 6$). (f) Inflammatory cell infiltration analysed by HE staining (scale bar = 20 μm, $n = 6$). (g) mRNA levels of proinflammatory cytokines in murine gingival tissue ($n = 6$). (h) Total bacterial load of ligatures on Day 0 (control) and Day 7 (periodontitis) after ligation ($n = 10$). Data are representative of two independent experiments. Data are presented as the mean ± SEM. Statistical significance was determined by unpaired, two-tailed Student's *t* tests. ABC, alveolar bone crest; CEJ, cementoenamel junction; WT, wild type

Ccr2$^{-/-}$ group increased by an average of 5.82% compared with that of the WT group (Figure 2d). Consistently, osteoclasts around the alveolar bone were significantly reduced in the knockout mice (Figure 2e). Haematoxylin and eosin (HE) staining demonstrated that in the WT mice, the soft tissue showed more severe damage, and the infiltrating inflammatory cells were deeper (Figure 2f). The mRNA levels of the proinflammatory cytokines CCL2, TNF-α, IL-1β, and IL-6 were significantly reduced in the gingival tissue of the *Ccr2*$^{-/-}$ mice, while the expression of IL-17 was not significantly different between the two groups (Figure 2g). There were no significant differences in total oral bacterial load between the two groups regardless of on Day 0 or Day 7 after ligature placement (Figure 2h), indicating that CCR2

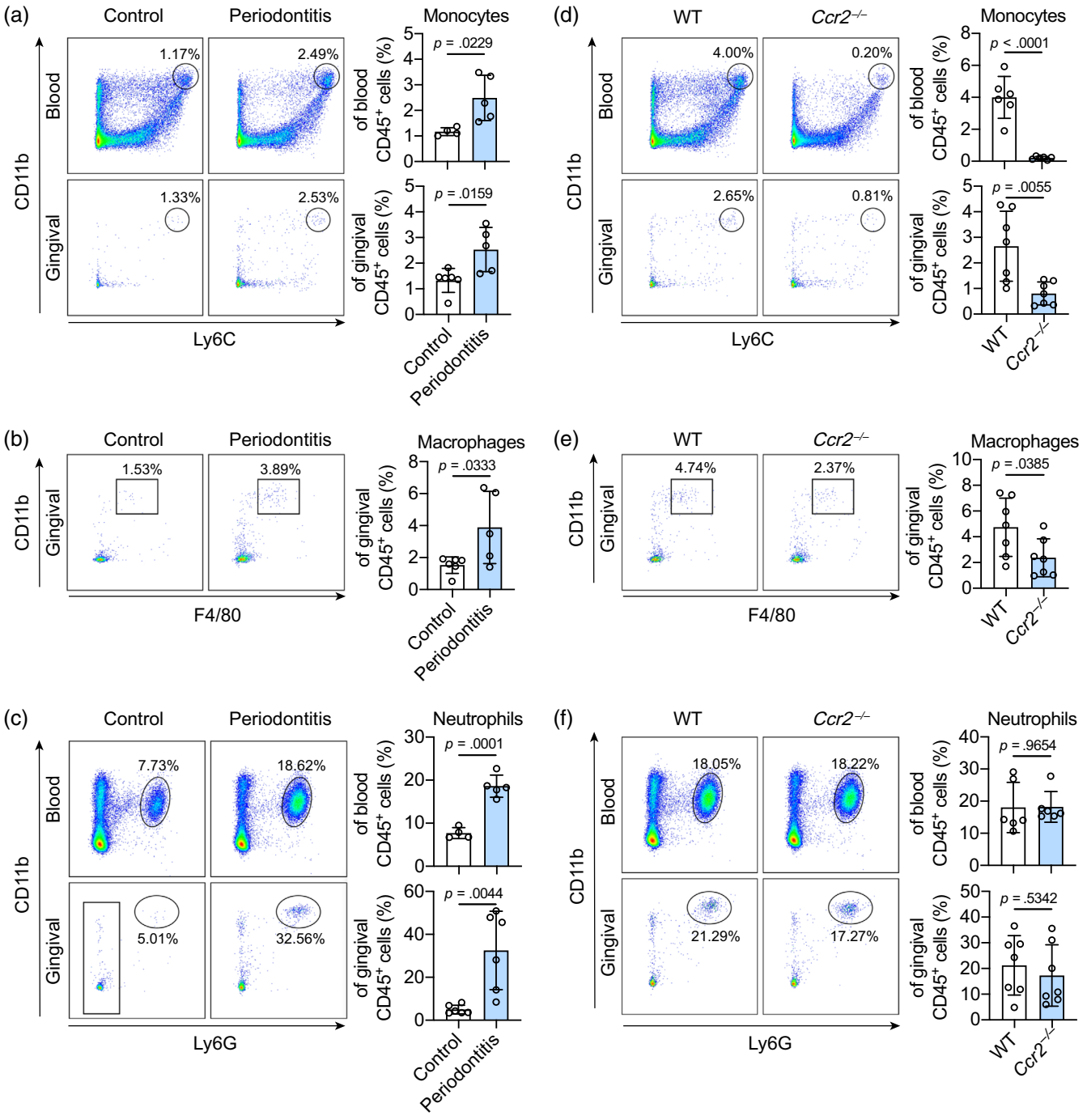

FIGURE 3 CCR2 deficiency suppresses the infiltration of monocytes and macrophages in the periodontium. (a) Representative flow cytometric images and statistical analysis of monocytes ($CD45^+CD11b^+Ly6G^-Ly6C^{hi}$) in peripheral blood and gingival tissues of the mice in the control and periodontitis groups. (b) Representative flow cytometric images and statistical analysis of macrophages ($CD45^+CD11b^+Ly6G^-F4/80^+$) in gingival tissues of the mice in the control and periodontitis groups. (c) Representative flow cytometric images and statistical analysis of neutrophils ($CD45^+CD11b^+Ly6G^+$) in peripheral blood and gingival tissues of the mice in the control and periodontitis groups. (d) Representative flow cytometric images and statistical analysis of monocytes in peripheral blood and gingival tissues of the WT and *Ccr2*$^{-/-}$ mice. (e) Representative flow cytometric images and statistical analysis of macrophages in gingival tissues of the WT and *Ccr2*$^{-/-}$ mice. (f) Representative flow cytometric images and statistical analysis of neutrophils in peripheral blood and gingival tissues of the WT and *Ccr2*$^{-/-}$ mice. Each sample contained gingival tissues from two mice, $n = 5$ or 6. Data are representative of two independent experiments. Data are presented as the mean ± SEM. Statistical significance was determined by unpaired, two-tailed Student's *t* tests

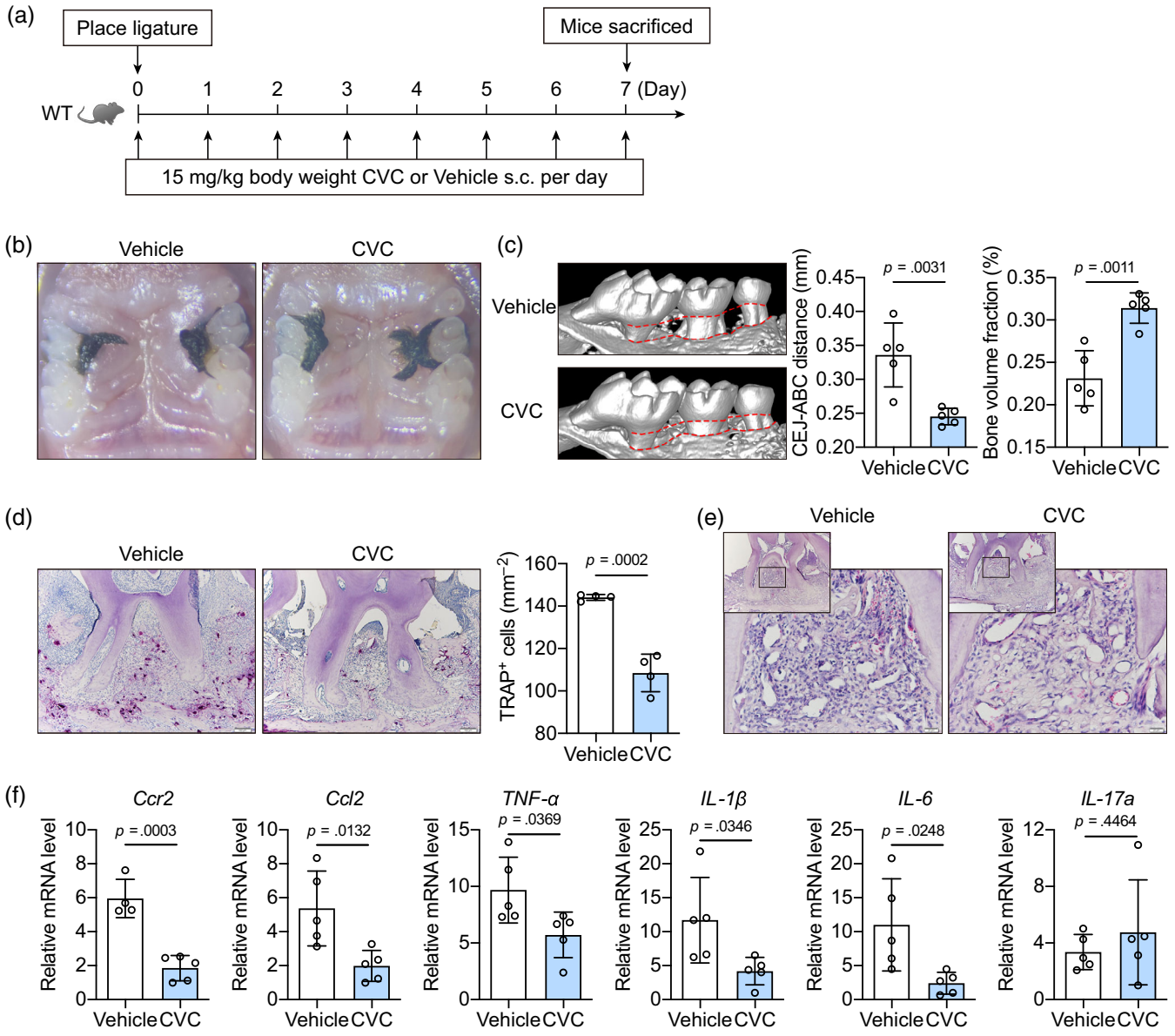

FIGURE 4 Systemic and preventive administration of CVC alleviates periodontal inflammation and alveolar bone loss in mice with periodontitis. (a) Schematic diagram of the experimental design. (b) Intra-oral images of mice were taken with a stereomicroscope. (c) Bone loss and bone volume fraction were analysed by micro-CT. (d) Number of osteoclasts per square millimetre was determined by TRAP staining (scale bar = 100 μm). (e) Inflammatory cell infiltration was analysed by HE staining (scale bar = 20 μm). (f) mRNA levels of proinflammatory cytokines in murine gingival tissues. $n = 5$ mice for each group. Data are representative of two independent experiments. Data are presented as the mean ± SEM. Statistical significance was determined by unpaired, two-tailed Student's *t* tests. ABC, alveolar bone crest; CEJ, cementoenamel junction; s.c., subcutaneous injection

knockout has no impact on oral microbes. Moreover, CCR2 deficiency did not impair systemic resistance to infection in the mice (Figure S3).

3.3 | CCR2 deficiency suppresses the infiltration of monocytes and macrophages in the periodontium

To investigate the composition of immune cells in peripheral blood and periodontal tissues, flow cytometric analysis was used. Periodontitis induced a significant increase in monocytes in peripheral blood and gingiva (Figure 3a), macrophages in gingiva (Figure 3b), neutrophils in peripheral blood and gingiva (Figure 3c). CCR2 deficiency markedly inhibited the migration of monocytes into circulation and the infiltration of monocytes and macrophages in gingival tissues caused by periodontitis (Figure 3d–e). However, the proportions of neutrophils in peripheral blood and gingival tissues were not affected by CCR2 knockout (Figure 3f).

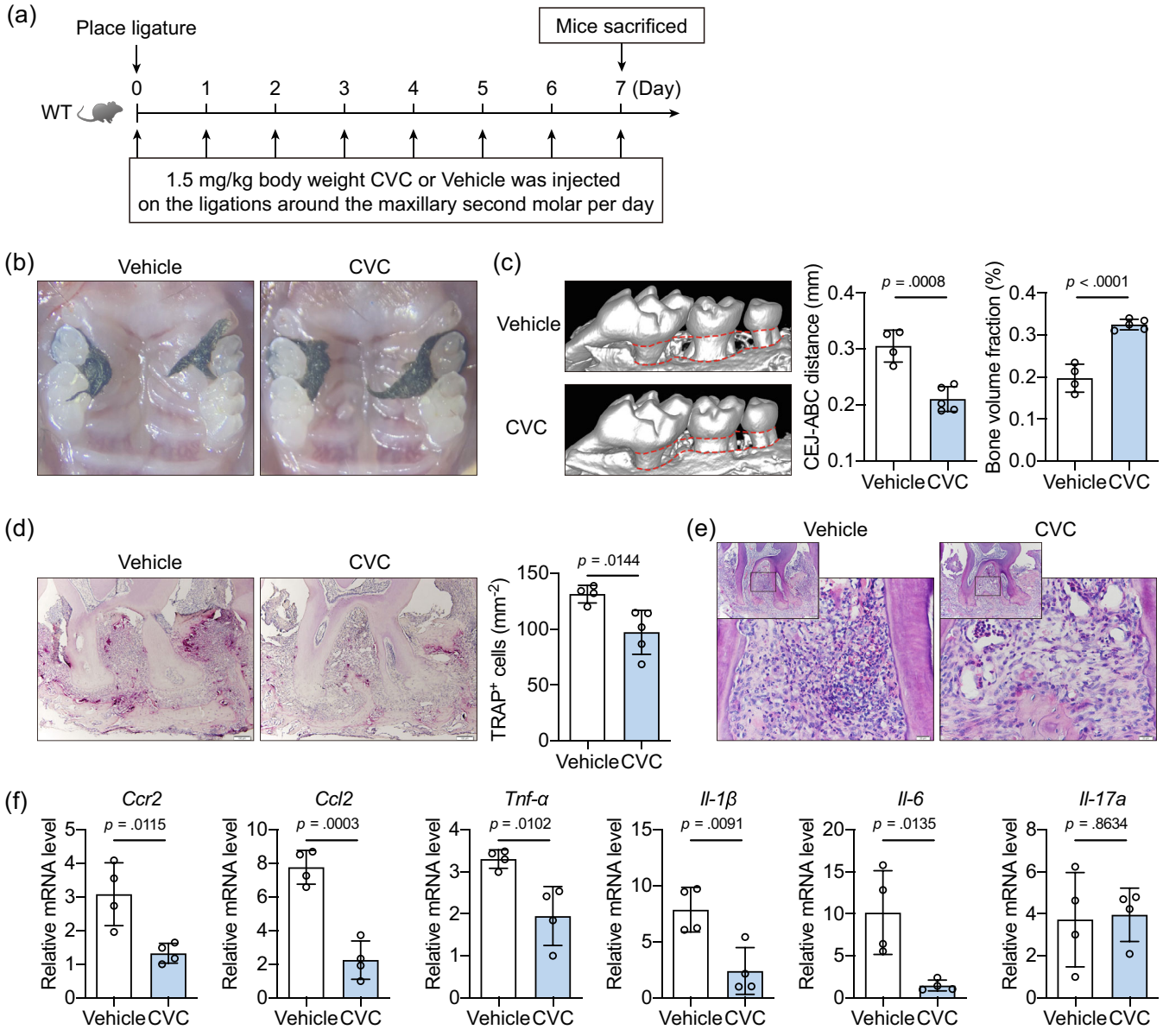

FIGURE 5 Local and preventive administration of CVC alleviates periodontal inflammation and alveolar bone loss in mice with periodontitis. (a) Schematic diagram of the experimental design. (b) Intra-oral images of mice were taken with a stereomicroscope. (c) Bone loss and bone volume fraction were analysed by micro-CT. (d) Number of osteoclasts per square millimetre was analysed by TRAP staining (scale bar = 100 μm). (e) Inflammatory cell infiltration analysed by HE staining (scale bar = 20 μm). (f) mRNA levels of proinflammatory cytokines in murine gingival tissues. CVC group, $n = 5$; vehicle group, $n = 4$. Data are representative of two independent experiments. Data are presented as the mean ± SEM. Statistical significance was determined by unpaired, two-tailed Student's *t* test. ABC, alveolar bone crest; CEJ, cementoenamel junction

3.4 | Preventive administration of CVC alleviates periodontal inflammation and bone loss in periodontitis mice

To investigate whether CVC could be used to prevent periodontitis, the experimental mice were divided into systemic administration groups (Figure 4a) and local administration groups (Figure 5a). Systemic treatment with CVC resulted in significant protection against periodontitis. The most intuitive manifestation was that gingival swelling in the CVC-treated group was milder than that in the vehicle-treated group (Figure 4b). Micro-CT analysis demonstrated that CVC prevented pathological absorption of alveolar bone of the maxillary second molars in the mice (Figure 4c). TRAP staining further confirmed the reduction in multinuclear osteoclasts in the alveolar bone region (Figure 4d). The inflammatory infiltration of the periodontium, estimated by HE staining, was significantly milder in the CVC-treated mice than in the vehicle-treated mice (Figure 4e). The expression of CCR2 and other proinflammatory cytokines (CCL2, TNF-α, IL-1β, and

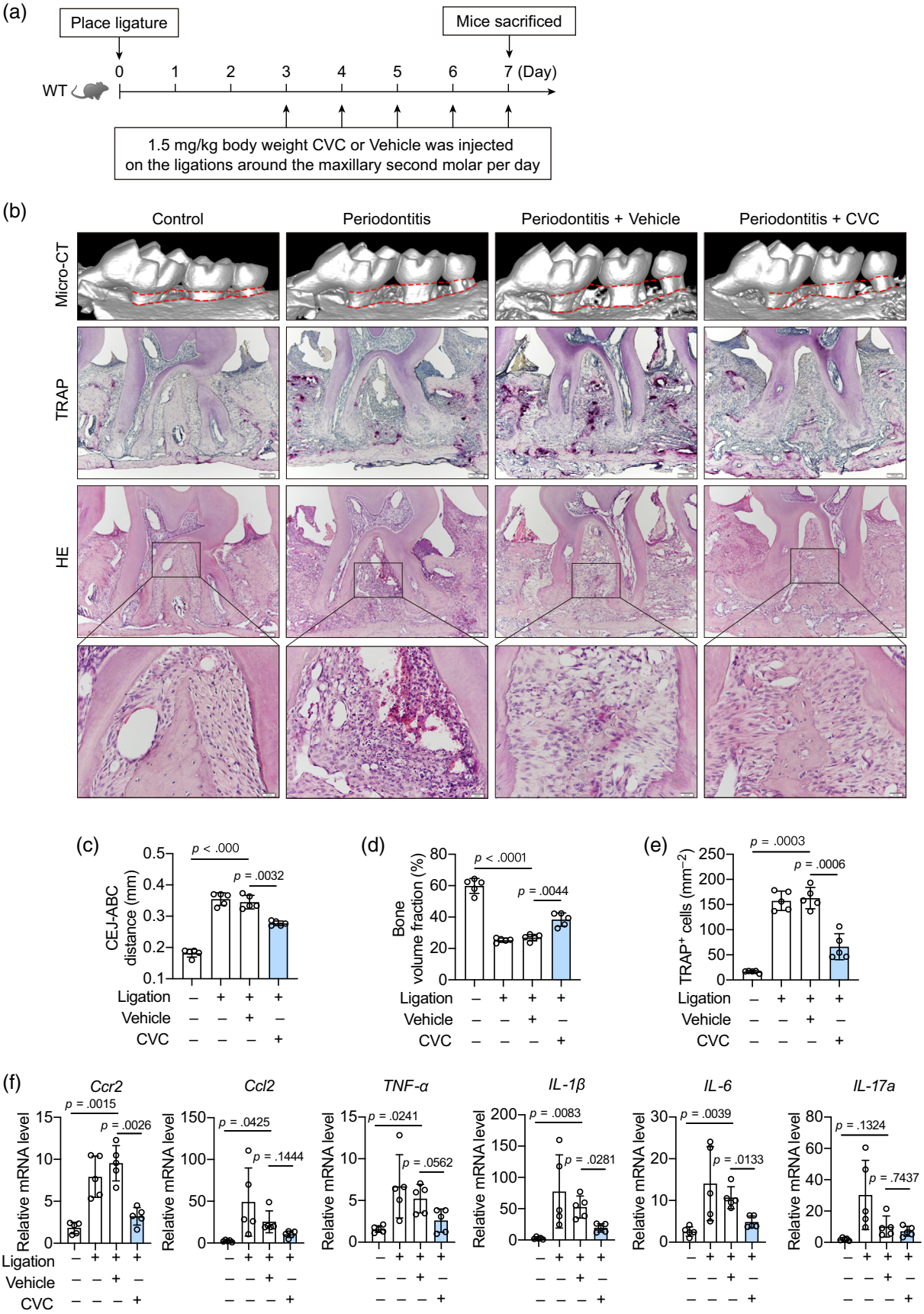

FIGURE 6 Legend on next page.

IL-6) was significantly decreased, which further confirmed the inhibitory effect of CVC on ligature-induced periodontitis (Figure 4f).

Topical administration of CVC also showed a favourable prophylactic effect in experimental periodontitis. Compared with the vehicle-treated group, the CVC-treated group showed milder gingival swelling (Figure 5b). Micro-CT scanning revealed less alveolar bone absorption and more residual bone volume in the CVC-treated mice (Figure 5c). The histological results showed that local administration of CVC significantly reduced the number of TRAP-positive osteoclasts (Figure 5d) and infiltrating inflammatory cells (Figure 5e) in the periodontal supporting tissues. Moreover, the expression of proinflammatory cytokines CCL2, TNF-α, IL-1β, and IL-6 was also inhibited by CVC (Figure 5f).

3.5 | Therapeutic administration of CVC attenuates periodontal inflammation and bone loss in periodontitis mice

To investigate the effect of CVC on preventing further progression of existing periodontitis, we designed an experimental protocol in which drug interventions were carried out after the onset of periodontitis (Figure 6a). Compared with the vehicle-treated group, the bone loss of the CVC-treated group was reduced by approximately 20% (Figure 6b-upper,c–d). Correspondingly, the histological results showed that the number of TRAP+ osteoclasts per unit area (Figure 6b-middle,e) and the degree of inflammatory infiltration also decreased significantly (Figure 6b-below). Real-time qPCR analysis revealed that the expression of CCR2, CCL2, TNF-α, IL-1β, and IL-6 was inhibited by local treatment with CVC, but the expression of IL-17A was not affected by this treatment (Figure 6f).

4 | DISCUSSION

Monocytes/macrophages play an important role in the pathogenesis of periodontitis (Jakubzick et al., 2017; Lira-Junior et al., 2020), while CCL2-CCR2 is the major signal responsible for the recruitment of monocytes/macrophages (Serbina et al., 2008). In addition, CCR2 was reported as an important participant in inflammatory diseases and bone metabolic diseases (Binder et al., 2009; Koide et al., 2010; Raghu et al., 2017), and periodontitis is a typical chronic inflammatory disease with abnormal bone remodelling. Therefore, we hypothesized that CCR2 may play an important role in the development of periodontitis and might be a potential therapeutic target. Our findings indicate that CCR2 participates in the progression of periodontitis by promoting monocytes/macrophages recruitment, proinflammatory cytokines production, and osteoclasts formation, and CVC may be a therapeutic drug for periodontitis (Figure 7).

Inflammation is one of the most important clinical manifestations of periodontitis. Periodontal pathogens induce the recruitment of immune cells, which release abundant proinflammatory cytokines, such as CCL2, TNF-α, IL-1β, IL-6, and IL-17A, in the process of pathogen killing, causing an inflammatory response that leads to tissue destruction (Koide et al., 2010; Hajishengallis et al., 2020). CCL2 can be produced by endothelial cells, gingival fibroblasts, monocytes, and macrophages in the periodontium (Hanazawa et al., 1993; Yu & Graves, 1995). Microbial stimuli induce periodontal tissue cells to rapidly release CCL2 (Serbina et al., 2008). Recruited monocytes/macrophages subsequently produce CCL2, TNF-α, IL-1β, and IL-6 to enhance the inflammatory response (Hanazawa et al., 1993; Jurdziński et al., 2020; Papathanasiou et al., 2020). Consistent with the detection results of human samples, the expression levels of the proinflammatory cytokines CCL2, TNF-α, IL-1β, and IL-6 in gingival tissues were elevated in the mice with periodontitis and were inhibited when CCR2 was deleted, indicating that CCR2 is the key target of periodontitis-induced inflammation.

Alveolar bone resorption, which is mainly caused by excessive formation and activation of osteoclasts, is another of the most distinctive clinical manifestation of periodontitis (Koide et al., 2010). The fusion and multinucleation of macrophages are essential for the formation of osteoclasts (Yao et al., 2021). Depleting monocytes/macrophages or suppressing inflammatory responses of monocytes/macrophages can effectively alleviate alveolar bone absorption in periodontitis (Lam et al., 2014; Wang et al., 2021). In osteoarthritis and osteoporosis, CCR2 was reported to be essential for the formation of osteoclasts, and CCR2 deficiency resulted in an elevated bone mass phenotype by reducing the numbers and activity of osteoclasts (Binder et al., 2009; Raghu et al., 2017). Corresponding to these publications, our data showed that CCR2 deficiency markedly reduced not only the proportion of monocytes/macrophages in gingival tissues but also the number of TRAP+ osteoclasts and alleviated alveolar bone resorption. Moreover, previous studies have reported that cytokines such as TNF-α, IL-1β, and IL-6 are involved in the destruction of alveolar bone (Koide et al., 2010; Sima & Glogauer, 2013; Okamoto et al., 2017). TNF-α can strongly promote IL-1β and IL-6 production. TNF-α, IL-1β, and IL-6 further promote osteoclastogenesis and osteoclast activation. Our results showed that periodontitis-induced elevation of TNF-α, IL-1β, and IL-6 expression was inhibited after CCR2 knockout. These results demonstrated that CCR2 signalling promotes osteoclast formation, alveolar bone resorption, and

FIGURE 6 Local and therapeutic administration of CVC alleviates periodontal inflammation and alveolar bone loss in mice with periodontitis. (a) Schematic diagram of the experimental design. (b) Upper: Three-dimensional reconstruction images of micro-CT scanning. Middle: TRAP staining images of maxillae sections (scale bar = 100 μm). Below: HE staining analysis of inflammatory cell infiltration (scale bar = 100 μm or 20 μm). (c) Statistical analysis of periodontitis-induced bone loss. (d) Statistical analysis of residual bone volume. (e) Statistical analysis of osteoclast numbers around alveolar bone. (f) mRNA levels of proinflammatory cytokines in murine gingival tissues analysed by real-time qPCR. $n = 5$ mice for each group. Data are representative of two independent experiments. Data are presented as the mean ± SEM. Statistical significance was determined by one-way analysis of variance with Brown–Forsythe and Welch test. ABC, alveolar bone crest; CEJ, cementoenamel junction

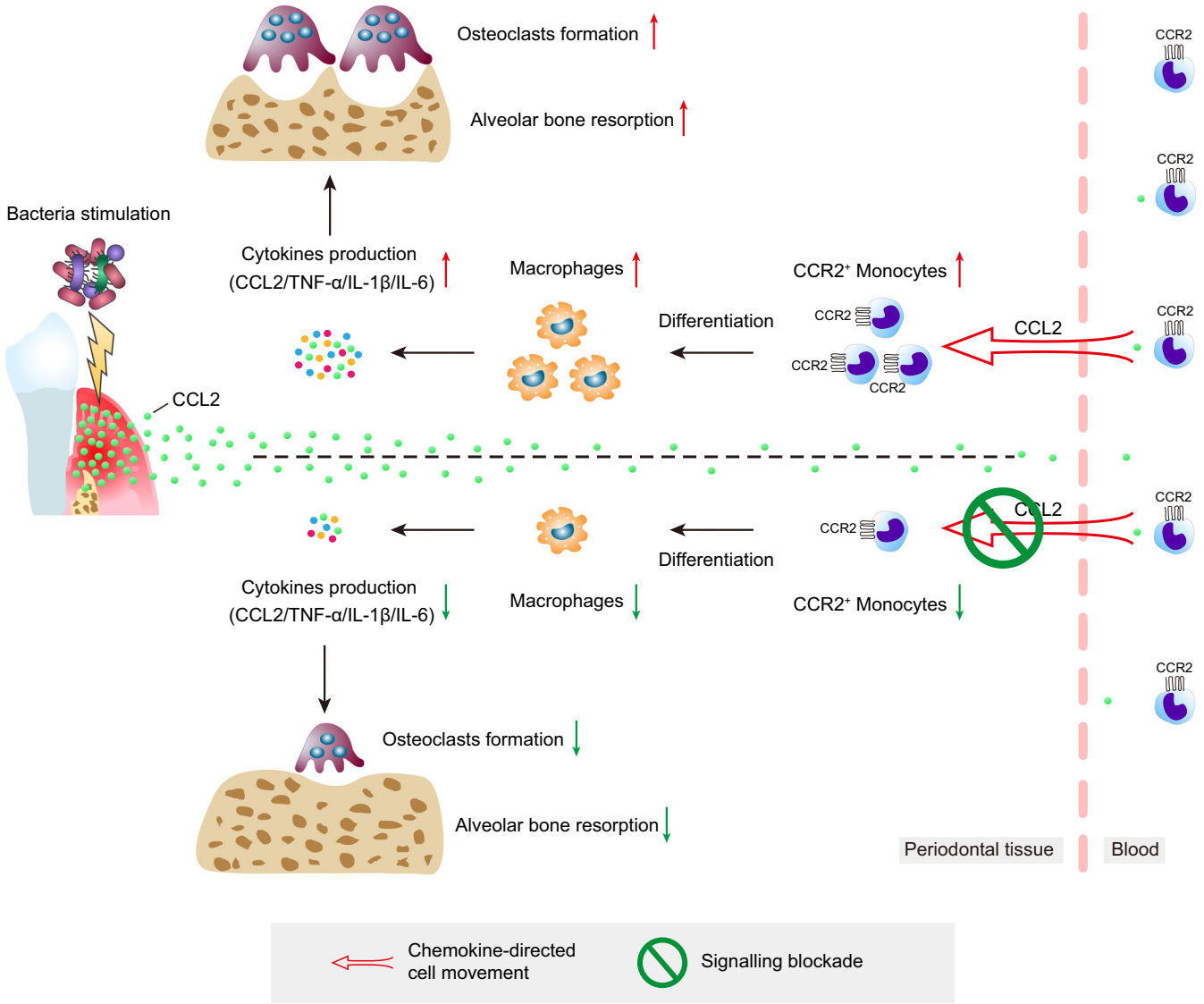

FIGURE 7 The mechanism diagram shows that CCR2 participated in the development of periodontitis through monocyte/macrophages recruitment

inflammatory destruction by affecting the migration and infiltration of monocytes/macrophages and the production of TNF-α, IL-1β, and IL-6.

CVC is a CCR2/CCR5 dual antagonist. In the present study, we confirmed the important role of CCR2 in promoting inflammation and alveolar bone absorption of periodontitis. CCR5 is also an important chemokine receptor that mediates the occurrence and development of periodontitis. Compared with healthy individuals, CCR5 was up-regulated in gingival crevicular fluid and gingival tissues of periodontitis patients, and decreased significantly after treatment (Gamonal et al., 2001; Garlet et al., 2003). In animal studies, CCR5 KO mice showed a lower periodontitis severity when compared with the WT strain (Repeke et al., 2010; Ferreira et al., 2011). Our data showed that CVC presented a good preventive and therapeutic effect on periodontitis, which may be due to its inhibition of CCR2 and CCR5 simultaneously. Another important rationale for our choice of CVC as a pharmaceutical inhibitor was that CVC has entered phase IIb clinical trial for the treatment of human immunodeficiency virus infection (Thompson et al., 2016) and phase III clinical trial for the treatment of steatohepatitis and liver fibrosis (Anstee et al., 2020), which suggests that CVC is of important clinical value and good safety. In addition, compared with developing a new drug from scratch, it is also of great social significance to tap more therapeutic potentials of existing drugs.

It is reported that IL-17A is also an important participant in the pathogenesis of periodontitis (Hajishengallis et al., 2016; Abusleme & Moutsopoulos, 2017). Consistent with these studies, our findings showed that the expression of IL-17A in the gingival of patients and mice with periodontitis is significantly up-regulated. However, there was no significant linear correlation between the expression of IL-17A and CCR2 in human gingival. And in mice, knockout of CCR2 or inhibition of CCR2/5 has no significant effect on the expression of IL-17A. Moreover, the regulatory network between CCR2/5 and IL-17 has never been reported. Therefore, we hypothesized that IL-17A is involved in a CCR2/5-independent regulatory mechanism in

periodontitis and that targeting CCR2 therapy alone may not be sufficient to improve the symptoms of patients with high levels of IL-17A.

This work was the first to thoroughly explore the relationship between CCR2 and periodontitis. However, the human samples from the healthy and periodontitis group were not matched for age and gender. Thus, more samples need to be collected to adjust the age and gender, and more well-designed clinical studies are encouraged to provide valuable information about the relevance between CCR2 and the severity of periodontitis in the future.

5 | CONCLUSION

Collectively, CCR2 plays a critical role in the development of periodontitis and CVC is a potential drug for the prevention and treatment of periodontitis.

AUTHOR CONTRIBUTIONS

Wenting Jiang, Ying Wang, and Wenjie Hu designed the study. Wenting Jiang and Tao Xu performed most of the experiments and analysis. Shasha Yuan, Zhanming Song, Qingqing Li, Shaoping She, Xuekang Wang, Yiping Wei, Meng Shi, Siqi Li, Zhongtian Liu, Yaqian Mo, and Ping Lv performed some experiments. Tao Xu, Cui Wang, Gang Yang, Jie Cao, and Fei Sun provided human specimens. Yu Zhang designed and performed the generation of CCR2 knockout mice. Wenting Jiang, Ying Wang, Wenjie Hu, and Yiping Wei prepared the manuscript with input from all authors.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

All data are available in the main text or the supplementary materials.

ETHICS STATEMENT

The present study involved clinical samples, which has been approved by the Ethics Committee of the Peking University Health Science Center (PKUSSIRB-201310068a). All animal experiments were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals and with the approval of the Ethics Committee of Peking University Health Science Center (LA2021494).

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