Characterisation of Innate Lymphoid Cells in Human and Murine Periodontitis

Wen Min ZENG[1#], Zong Xian LI[2#], Chao ZHONG[2], Yu Feng XIE[1], Xiao Qian YU[3]

Objective: *To characterise the subset dynamics of innate lymphoid cells (ILCs) in human and murine periodontitis to elucidate their roles in disease progression.*
Methods: *Human gingival samples were harvested from periodontitis patients and healthy controls. A murine ligature-induced periodontitis model was established. Gingival ILC subsets (ILC1s, ILC2s, ILC3s) were analysed by flow cytometry.*
Results: *Human periodontitis exhibited elevated total ILCs (from 1.34% to 2.06%) and a pronounced increase in ILC3s (from 0.92% to 29.70%). In mice, 3 days post-ligation, there was a marked decrease in total ILCs (from 10.80% to 0.85%), primarily due to the depletion of ILC2s (from 81.55% to 60.08%), whereas ILC1s (from 1.41% to 15.07%) and ILC3s (from 0.75% to 2.87%) expanded. These changes became even more pronounced 7 days after ligation.*
Conclusion: *Species-specific ILC dynamics underscore distinct immunopathogenic mechanisms: human periodontitis is characterised by the predominance of ILC3s, whereas, in murine disease, there is an association with the loss of ILC2s and an expansion of pro-inflammatory ILC1s/ILC3s. These observations place ILC3s as central mediators in chronic human diseases and emphasise the necessity for therapeutic strategies tailored to target specific ILC subsets.*
Keywords: *innate immunity, innate lymphoid cells, murine ligature-induced periodontitis, periodontitis*
Chin J Dent Res 2026;29(3):227–235; doi: 10.3290/j.cjdr.b7093222

1 Department of Periodontology, Shanghai Stomatological Hospital & School of Stomatology, Fudan University, Shanghai Key Laboratory of Craniomaxillofacial Development and Diseases, Fudan University Shanghai, P.R. China
2 Department of Immunology, School of Basic Medical Sciences, NHC Key Laboratory of Medical Immunology, Medicine Innovation Center for Fundamental Researches on Major Immunology-Related Diseases, Peking University, Beijing, P.R. China
3 Department of Periodontology, Peking University School and Hospital of Stomatology, National Center of Stomatology, National Clinical Research Center for Oral Diseases, Beijing, P.R. China
# Equal contribution.

Corresponding authors: Dr Chao ZHONG, Department of Immunology, School of Basic Medical Sciences, Peking University, Beijing, P.R. China. Email: zhongc@pku.edu.cn;

Dr Yu Feng XIE, Department of Periodontology, Shanghai Stomatological Hospital & School of Stomatology, Fudan University, Shanghai, P.R. China. Email: yufengxie_123@163.com;

Dr Xiao Qian YU, Department of Periodontology, Peking University School and Hospital of Stomatology, Beijing, P.R. China. Email: y_pk@163.com.

This work was supported by the Scientific Research Foundation of Shanghai Stomatological Hospital & School of Stomatology, Fudan University (SSH-2024-A04) and the National Natural Science Foundation of China (no. 81991500, 81991502, U23A20167, 32170896).

Periodontitis, a chronic inflammatory disease affecting the tooth-supporting apparatus, is a leading cause of tooth loss globally and is intricately linked to dysbiosis of the oral microbiota and dysregulated host immune responses.[1] Currently, the treatment of periodontitis mainly involves removal of the microbial biofilm, and there is no relevant intervention for dysregulated host immunity.[2,3] This gap underscores the urgent need to elucidate the immunopathogenic mechanisms driving periodontitis, particularly those involving innate immune cells.

Innate lymphoid cells (ILCs), a growing class of innate immune cells, imitate the appearance and abilities of T cells but lack adaptive antigen receptors.[4] ILCs are classified mainly as Group 1 (ILC1s and natural killer cells), Group 2 (ILC2s) and Group 3 (ILC3s and lymphoid tissue inducer cells) based on their signature cytokines and transcription factors.[5,6] ILCs, which are mostly tissue-resident cells, are frequently found on the mucosal surfaces. They play key functions in controlling and resolving inflammation, and variations in their proportion are linked to various pathological diseases

including cancer and gastrointestinal, pulmonary and skin diseases.[7-9]

Several studies have reported that ILCs are present in oral mucosa and involved in human periodontitis.[10-13] However, critical questions remain unresolved: Do ILC subset dynamics differ between humans and experimental models, and how do these cells contribute to disease progression or resolution? This study aims to characterise ILC subset distribution and functional changes in both murine ligature-induced periodontitis and human periodontitis. By integrating flow cytometric analysis of gingival tissues from human and mice, the present authors seek to define species-specific ILC dynamics, correlate these changes with alveolar bone loss and identify potential therapeutic targets within the ILC compartment. By bridging human and murine data, this study provides a foundation for future mechanistic studies and immunomodulatory interventions aimed at restoring immune balance in periodontitis.

Materials and methods

Human gingival sample collection

The study protocol was approved by the Review Board and Ethics Committee of Peking University Health Science Center (approval no. PKUS-SIRB-2013017) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from each participant before inclusion in the study.

All participants were recruited from the department of Periodontology at Peking University School and Hospital of Stomatology. Patients were excluded from the study if they met one or more of the following criteria: a history of systemic diseases, including pregnant women; intake of anti-microbial medication or anti-inflammatory drugs or periodontal treatment within the last 6 months; and smoking. Healthy gingival samples were obtained from periodontally healthy individuals undergoing crown lengthening surgery. The inclusion criteria in this group were periodontally healthy with probing depth (PD) ≤ 3 mm, no bleeding on probing, no clinical attachment loss or gingival recession, no mobility and furcation involvement, and continuous and clear lamina dura with no widened periodontal space revealed from radiographic examination. Inflammatory gingival samples were collected from patients with periodontitis (stage III to IV) undergoing periodontal flap surgery. An internal bevel incision, approximately 2 to 3 mm in depth and 5 to 7 mm in length, was made along the gingival margin of teeth with PD ≥ 6 mm and bleeding index ≥ 2. Then, the tissues were harvested using a Gracey curette. After excluding subjects with a history of systemic disease, anti-microbial medication, anti-inflammatory drugs or periodontal treatment within the last 6 months, six periodontitis patients and five healthy controls were finally selected.

Mouse model of experimental periodontitis

Six-week-old C57BL/6J male mice were purchased from the Charles River Laboratory (Beijing, China). All mice used in this study were bred and maintained in a specific-pathogen-free facility. Mice were randomly divided into three groups: no ligation (control), 3-day ligation (L3) and 7-day ligation (L7). For the ligation groups, after anaesthesia with sodium pentobarbital (6 mg/100 g, intraperitoneal injection), a 5-0 sterile silk ligature was placed around the bilateral maxillary second molars. Depending on the group, the silk ligatures were removed 3 or 7 days later, and the mice were sacrificed. Gingival tissues surrounding the maxillary molars were harvested and placed in ice-cold Roswell Park Memorial Institute (RPMI) 1640 (Gibco, Grand Island, NY, USA) for flow cytometry. Then, the maxillae were dissected carefully and fixed in 4% paraformaldehyde solution for 48 hours for bone loss analysis. All animal experimental procedures were approved by the Animal Welfare Ethics of Peking University Biomedical Ethics Committee (LA2022217).

Alveolar bone resorption measurements

For evaluation of periodontitis-induced bone loss, microcomputed tomography (microCT) analysis was conducted. As described in a previous study,[4] the paraformaldehyde-fixed maxilla was scanned using a high-resolution microCT system (Inveon MM Gantry-STD 3121, Siemens, Germany). The alveolar bone loss was evaluated based on the distance from the alveolar bone crest to the cementoenamel junction (ABC-CEJ) on the tooth, which was measured as previously described.[15]

Cell preparation

Gingival samples from mice and humans were cut into 1- to 2-cm-long pieces and incubated for 30 minutes at 37°C in RPMI 1640 containing 0.5 mg/ml dispase (Sigma-Aldrich, St Louis, MO, USA). Then, the tissues were digested in RPMI 1640 containing 0.05 mg/ml collagenase IV (Sigma-Aldrich) and 0.1 mg/ml deoxyribonu-

clease (DNase) I (Sigma-Aldrich) in a shaker incubator at 37°C for 1 hour and during the last 5 minutes of digestion, 50 µl 0.5 M ethylenediaminetetraacetic acid (EDTA) was added. Finally, the digested tissues were filtered through a 40-µm filter, centrifuged at 540 g for 6 minutes and resuspended in phosphate-buffered saline (PBS) with 0.2% bovine serum albumin (BSA).

Flow cytometry

The single-cell suspensions were first stained with anti-CD16/CD32 (BioLegend, San Diego, CA, USA) antibody to block the Fc receptors. Antibodies for surface molecules and fixable viability dye were stained together for 30 minutes at 4°C. For transcription factor staining, the cells were fixed and permeabilised with a Foxp3 Staining Buffer Set (eBioscience, San Diego, CA, USA), then, transcription factors were stained for at least 4 hours at 4°C. Flow cytometry analyses were performed on an LSRFortessa (BD Biosciences, Franklin Lakes, NJ, USA). Data analysis was performed using FlowJo software (v10, BD Biosciences).

Antibodies specific to mice included anti-CD3e-FITC (145-2C11) (eBioscience), anti-CD19-FITC (eBio1D3) (eBioscience), anti-CD5-FITC (53-7.3) (eBioscience), anti-Gr-1-FITC (RB6-8C5) (eBioscience), anti-B220-FITC (RA3-6B2) (eBioscience), anti-CD127-BV786 (eBioRDR5) (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA), anti-CD45.2-PE (104) (BioLegend), anti-RORγt-PE-CF594 (Q31-378) (BD), anti-GATA3-PE-Cy7 (L50-823) (BD Biosciences) and anti-NKp46-BUV737 (29A1.4) (BioLegend).

Antibodies (BioLegend) specific to humans included anti-CD3-FITC (OKT3), anti-CD19-FITC (HIB19), anti-CD14-FITC (HCD14), anti-CD16-FITC, anti-CD138-FITC (HI49), anti-CD123-FITC (6H6), anti-CD34-FITC (581), anti-CD127-BV650 (AO19D5), anti-CD45-APC-Cy7 (2D1), anti-c-Kit-BV786 (104D2) and anti-CRTH2-PE-Dazzle594 (BM16).

Statistical analysis

The statistical significance of differences among groups was determined by independent two-tailed Student *t* tests or one-way analysis of variance (ANOVA) with GraphPad Prism 9 software (La Jolla, CA, USA). $P < 0.05$ was considered statistically significant. All data are presented as the mean and standard error of the mean (SEM).

Results

ILCs were increased in gingival tissues of human periodontitis

The gating strategy for human ILC cells was live $CD45^+$ lineage (CD3, CD19, CD14, CD16, CD138, CD123, $CD34)^-$ and $CD127^+$. ILC2s were defined by the expression of CRTH2, ILC3s are classified by the expression of c-Kit. ILC1s were currently described as $CRTH2^-$ $c\text{-}Kit^-$ ILCs (Fig 1a). The percentage of total ILCs in gingival tissues was significantly increased in periodontitis patients compared with healthy individuals (1.34% ± 0.18% vs 2.06% ± 0.18%, respectively, $P < 0.05$) (Fig 1b and c). These findings indicate that periodontitis is associated with an expanded ILC compartment.

ILC3s were increased in human periodontitis

In healthy controls, ILC1s constituted 90.80% of total ILCs, ILC2s 8.27% and ILC3s 0.92%. In periodontitis patients, ILC1s accounted for 69.27%, ILC3s 29.70% and ILC2s 0.89% (Fig 2a). The percentage of ILC3s in total ILCs was significantly higher in periodontitis patients compared to healthy controls ($P < 0.01$), while ILC1s showed a significant decrease ($P < 0.01$) (Fig 2b). Within the $CD45^+$ immune cell population, the percentage of ILC3s was significantly elevated in periodontitis patients ($P < 0.01$), whereas ILC1s showed a non-significant trend towards increase (Fig 2c). ILC2s showed no significant differences between the two groups. These findings indicate that periodontitis is associated with an altered distribution of ILC subsets, particularly an increase in ILC3s.

MicroCT analysis

To characterise ILC subset dynamics in murine periodontitis, a murine ligature-induced periodontitis model was established. Alveolar bone level was evaluated using microCT scanning. Periodontitis was successfully induced, with obvious bone loss around the maxillary molars observed after ligation (Fig 3). Compared to the control group, the mean ABC-CEJ distance was slightly increased in the L3 group (0.19 ± 0.01 mm vs 0.21 ± 0.01 mm, respectively; $P > 0.05$). A significant increase in this distance was observed in the 7-day ligation group (0.30 ± 0.01 mm; $P < 0.01$).

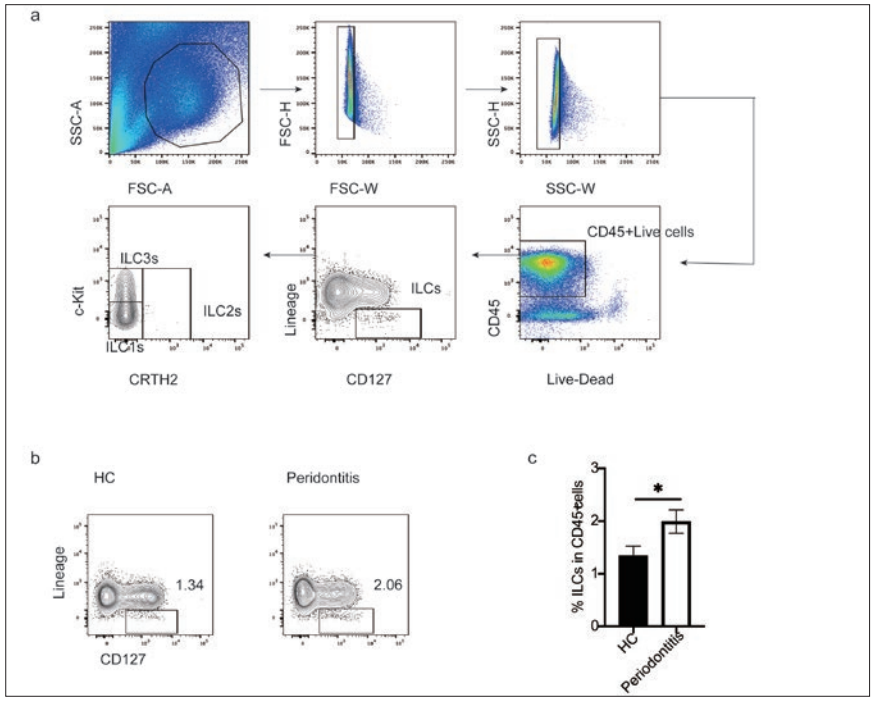

Fig 1a to c Increased ILCs in gingival tissues of human periodontitis. Gating strategy of total ILCs and ILC subsets. Lineage markers include CD3, CD19, CD14, CD16, CD138, CD123 and CD34 **(a)**. Representative flow cytometry analysis of total ILCs in human gingival tissues **(b)**. Frequency (in $CD45^+$ cells) of total ILCs **(c)**. HC, healthy control group. Data are shown as mean ± SEM. *$P < 0.05$.

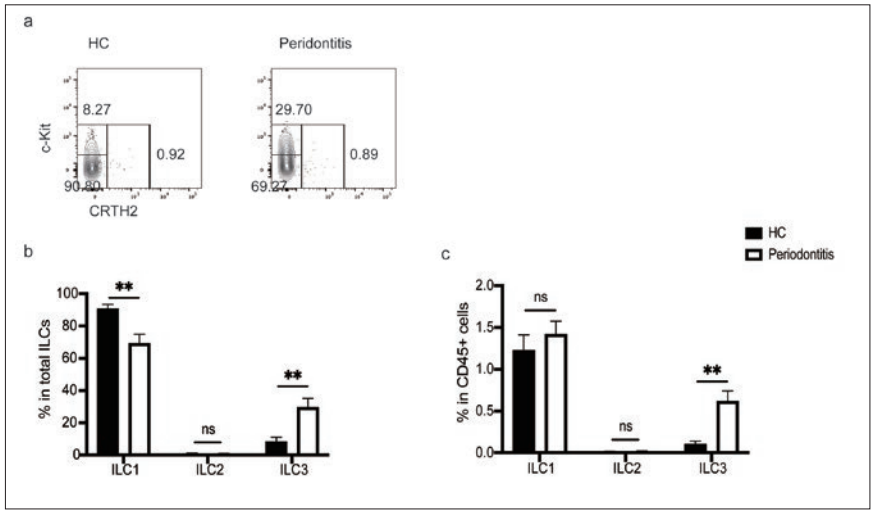

Fig 2a to c Distribution of distinct ILC subsets in human gingival tissues. Representative flow cytometry analysis of ILC1s, ILC2s and ILC3s in human gingival tissues **(a)**. Frequency in total ILCs **(b)** and in $CD45^+$ cells **(c)** of ILC1s, ILC2s and ILC3s in human gingival tissues. Data are shown as mean ± SEM. ns, no significance. **$P < 0.01$.

ILCs were decreased in gingival tissues of mice with periodontitis

The gating strategy of total ILCs and ILC subsets is shown in Fig 4a. ILC cells were live $CD45^+$ Lineage (CD3e, CD19, CD5, B220, Gr-1)$^-$ $CD127^+$. Subsequently, ILC1s were identified as $NKp46^+$ $ROR\gamma t^-$ $GATA3^-$ cells, ILC2s as $GATA3^+$ $NKp46^-$ $ROR\gamma t^-$ cells, and ILC3s as $ROR\gamma t^+$ $NKp46^-$ cells. An increase in lymphocyte infiltration was observed in the ligation groups (Fig 4b). Compared to the control group, the percentage of $CD45^+$ immune cells increased significantly after 3-day ligation (6.27% ± 0.46% vs 24.72% ± 3.86%, respectively; $P < 0.001$), with a further increase in the 7-day ligation group (41.77% ± 1.87%) (Fig 4c). Consistently, the absolute number of $CD45^+$ cells in the ligation groups was significantly higher than that in the control group (Fig 4d). The percentage of total ILCs was markedly decreased in the 3-day ligation group compared with the control group (0.85% ± 0.15% vs 10.80% ± 0.82%, respectively; $P < 0.001$). A further decrease was observed in the 7-day ligation group (0.54% ± 0.04%) (Fig 4e and f). There was no significant difference between the 3-day and 7-day ligation groups. This reduction in the percentage of ILCs was accom-

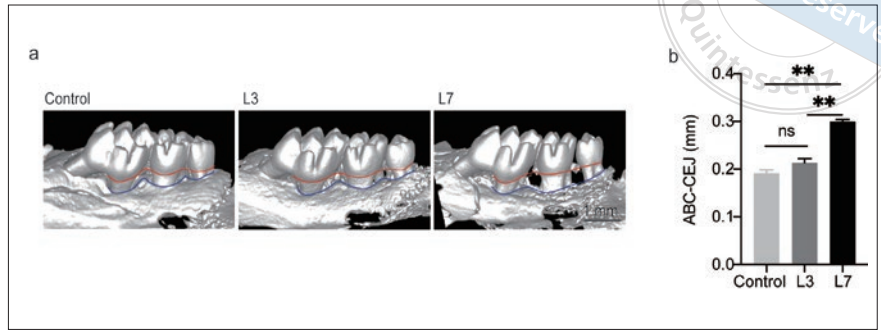

Fig 3a and b Alveolar bone loss induced by ligation. Representative 3D views of the maxillae by microcomputed tomography. Ligature-induced alveolar bone loss can be seen around the maxillary molars in both ligation groups **(a)**. Distance (in millimetres) from the alveolar bone crest to the cementoenamel junction (ABC-CEJ) **(b)**. Data are shown as mean ± SEM (n = 6 per group). L3, 3-day ligation group; L7, 7-day ligation group; ns, no significance. ***P* < 0.01.

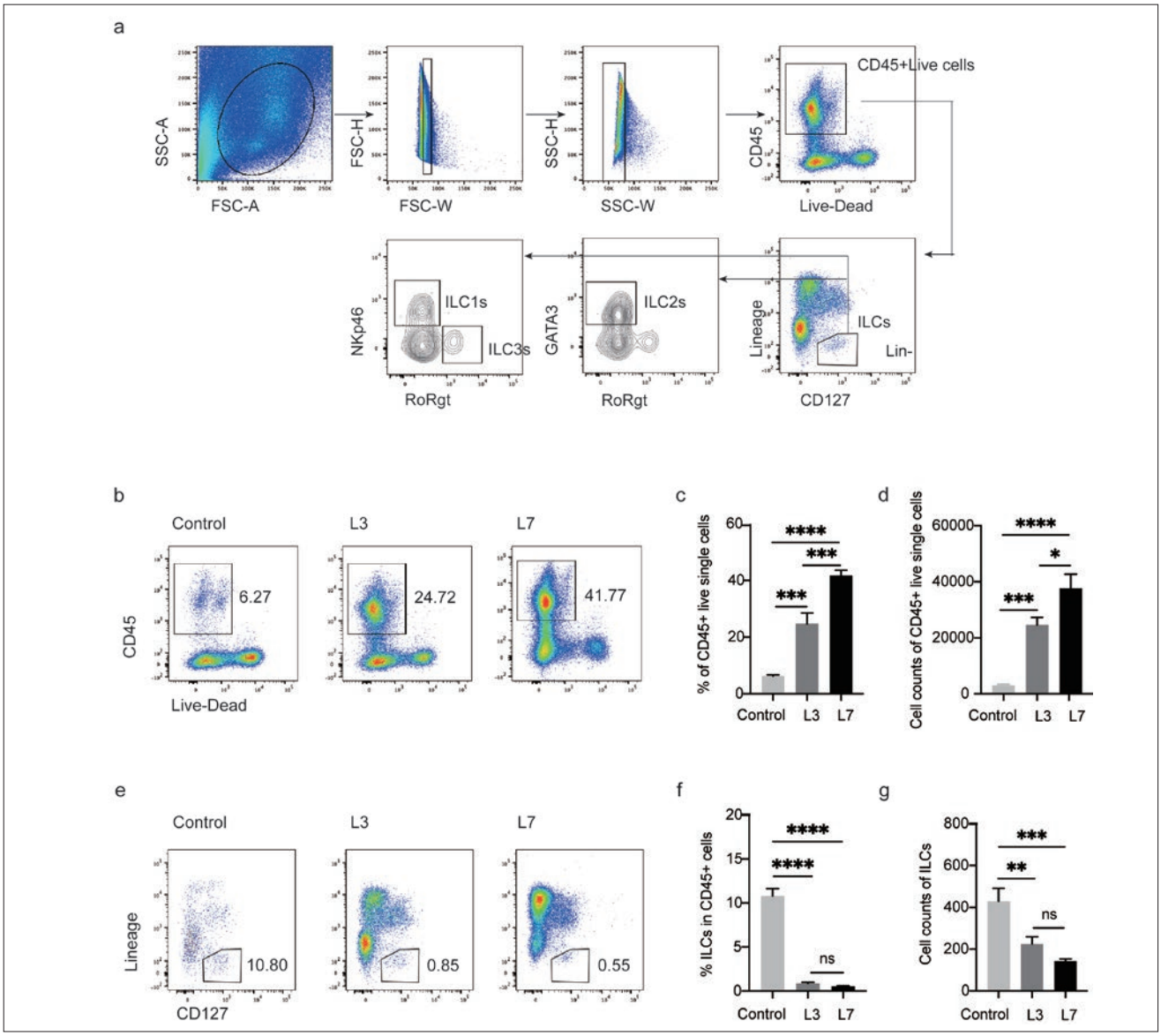

Fig 4a to g Decreased ILCs in gingival tissues of mice with periodontitis. Gating strategy of total ILCs and ILC subsets. Lineage markers include CD3e, CD19, CD5, B220 and Gr-1 **(a)**. Representative flow cytometry analysis of $CD45^+$ cells in gingival tissues of mice **(b)**. Frequency (in single cells) **(c)** and absolute cell numbers **(d)** of $CD45^+$ live cells in gingival tissues of mice. Representative flow cytometry analysis of total ILCs in gingival tissues of mice **(e)**. Frequency (in $CD45^+$ cells) **(f)** and absolute cell numbers **(g)** of total ILCs in gingival tissues of mice. Data are shown as mean ± SEM (n = 6 per group, gingiva from 3 mice were pooled as one sample). L3, 3-day ligation group; L7, 7-day ligation group; ns, no significance. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.

panied by a corresponding decrease in the absolute number of ILCs in the ligation groups as compared to the control group ($P < 0.01$) (Fig 4g).

Distribution of ILC subsets in gingival tissues of mice with periodontitis

Flow cytometric analysis revealed that compared to the control group, ligation significantly altered the percentages of ILC subsets, with ILC2s decreasing and ILC1s and ILC3s increasing (Fig 5). In the control group, ILC2s constituted the majority of total ILCs (81.55% ± 4.03%), followed by ILC1s (1.41% ± 0.20%) and ILC3s (0.75% ± 0.25%). After 3-day ligation, the proportion of ILC2s decreased to 60.08% ± 7.39%, whereas ILC1s increased to 15.07% ± 2.73% and ILC3s rose to 2.87% ± 0.32%. With 7-day ligation, ILC2s further dropped to 33.18% ± 2.85%, ILC1s reached 23.73% ± 2.99%, and ILC3s increased to 6.67% ± 1.56%. Additionally, absolute cell counts of ILC subsets were measured. For ILC1s, cell counts were significantly higher in 3-day ligation mice than in control mice ($P < 0.05$). There was an even more pronounced impact in the 7-day ligation group. In the case of ILC2s, both the 3-day and 7-day ligation groups exhibited reduced cell counts compared to the control group ($P < 0.001$); however, there was no significant difference between the 3-day and 7-day ligation groups. For ILC3s, the number of cells was notably greater in the 7-day ligation group compared with the control group ($P < 0.01$), whereas no significant difference was observed between the control and 3-day ligation groups.

Discussion

ILCs are tissue-resident cells primarily localised at mucosal barriers such as the gastrointestinal tract, airway and skin, which are constantly exposed to a plethora of triggers requiring immune control, including diverse commensal microbiome, ongoing damage from mastication and dietary and airborne antigens.[16-18] They have the unique capacity to initiate rapid responses against pathogens, provoked by changes in the cytokine profile of the respective tissue. Moreover, they regulate tissue inflammation and homeostasis.[19] ILC1s contribute to protective immune responses directed against intracellular bacteria or viruses by their IFN-γ production.[20,21] ILC2s are classically associated with tissue repair and response to infections with extracellular parasites or microbes through cytokines such as IL-5 and IL-13.[22] ILC3s, critical for mucosal barrier defence, are known to produce IL-22 and IL-17, cytokines that play an important role in regulating mucosal immunity and tissue homeostasis in the intestine and lungs.[23-25] However, the role of ILCs in the oral barrier remains poorly understood. This study reveals the dynamic distribution change of ILC subsets in oral mucosal inflammatory diseases such as periodontitis, highlighting their complex contributions to disease pathogenesis.

In this study, the present authors performed parallel profiling of ILCs in human and murine periodontitis. In mice, the ligature-induced acute model demonstrated a striking depletion of total ILCs, driven predominantly by the loss of ILC2s. ILC2s are the major subset within the ILCs compartment in gingival tissue from mice with/without periodontitis. The observed reduction in ILC2s may disrupt resolution pathways, exacerbating inflammation and enabling the expansion of pro-inflammatory ILC1s and ILC3s. In contrast, human periodontitis samples exhibited an overall increase in total ILCs. In line with earlier studies,[10,12] the present authors found that ILC1s were the most abundant ILC subset in gingival tissue from both healthy controls and periodontitis patients. ILC3s were found to be abnormally elevated in the gingival tissues of patients with periodontitis. This divergence from murine findings may reflect fundamental differences in disease chronicity and immune regulation. Human periodontitis is a chronic condition characterised by sustained microbial challenge and adaptive immune engagement,[2,26] which may favour the persistence of ILC3s. The dominance of ILC3s in human disease echoes observations in other chronic inflammatory disorders, such as inflammatory bowel disease, where ILC3s drive pathological inflammation.[8,27] IL-17, a primary cytokine secreted by ILC3s, has the capacity to amplify inflammation. It does so by excessively recruiting neutrophils, enhancing the production of pro-inflammatory cytokines and activating osteoclasts, all of which may contribute to immunopathology and bone destruction.[28,29] Dysregulated IL-17 production may play a pathogenic role in periodontitis through distinct yet potentially overlapping mechanisms.[30-32] The increase of ILC3s both in murine and human periodontitis suggests that ILC3/IL-17-driven inflammation may act as a key mediator in periodontitis bone loss. Notably, the reduction in ILC1s in human periodontitis contrasts with murine data, suggesting that human ILC1s may play a regulatory or compensatory role that diminishes as disease progresses. Notably, due to the definition of murine ILC subsets based on positive expression of NKp46, RORγt and GATA3, a fraction of ILCs (e.g., NKp46$^-$ RORγt$^-$ GATA3$^-$ or cells with non-canonical phenotypes) were not assigned to ILC1/2/3, leading to summed proportions below 100%. The proportions of

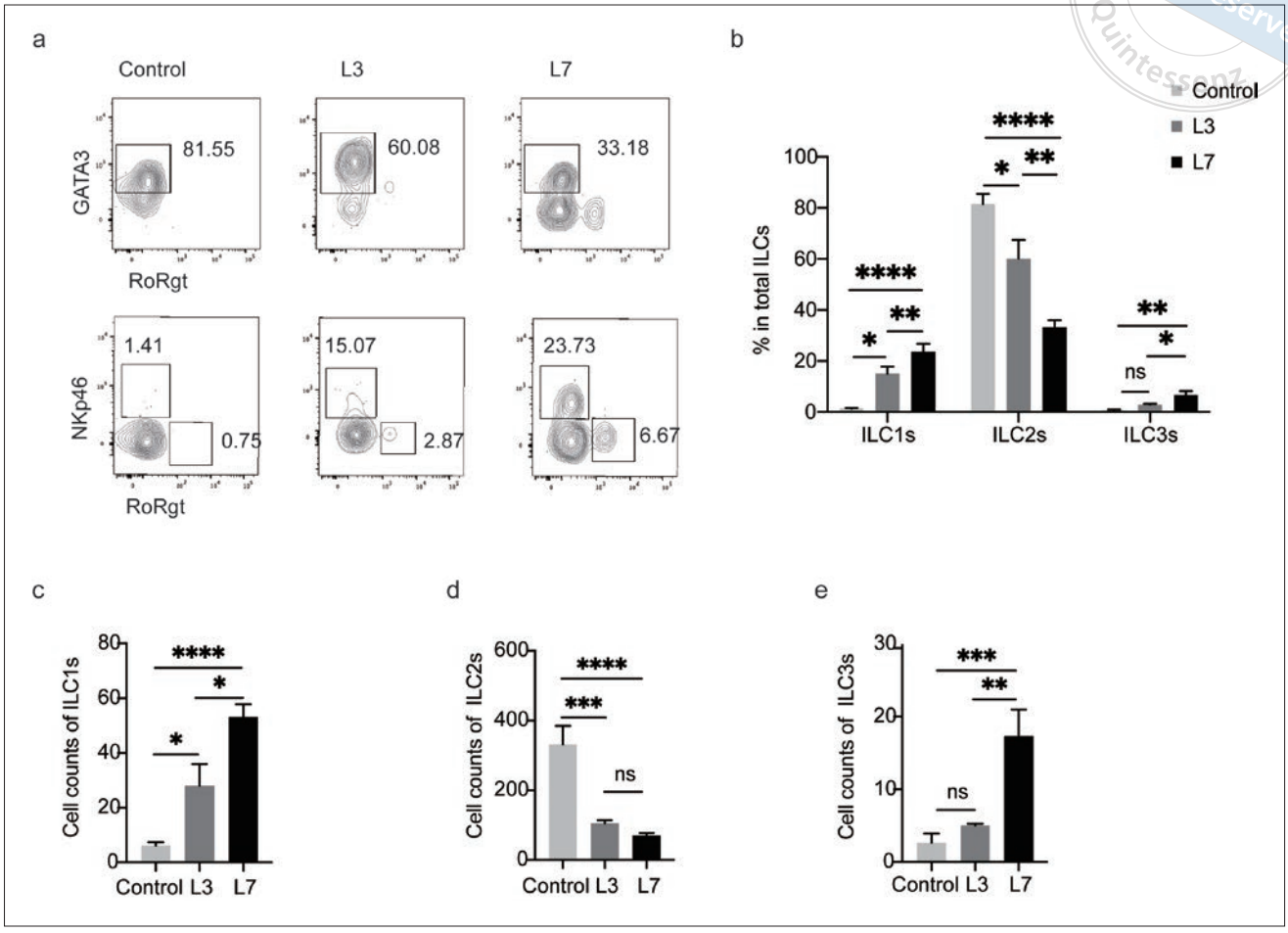

Fig 5a to e Distribution of distinct ILC subsets in gingival tissues of mice. Representative flow cytometry analysis of ILC1s, ILC2s and ILC3s in gingival tissues of mice **(a)**. Frequency (in total ILCs) of ILC1, ILC2 and ILC3 in gingival tissues of mice **(b)**. Absolute cell numbers of ILC1s **(c)**, ILC2s **(d)** and ILC3s **(e)** in gingival tissues of mice. Data are shown as mean ± SEM (n = 6 per group, gingiva from 3 mice were pooled as one sample). L3, 3-day ligation group; L7, 7-day ligation group; ns, no significance. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

unclassified ILCs were 16.29%, 21.98% and 36.42% in the control, 3-day ligation and 7-day ligation groups, respectively, suggesting enhanced ILC phenotypic plasticity during periodontitis.

The species-specific discrepancies highlighted in this study may also stem from methodological and biological factors. The murine ligature model induces rapid, localised inflammation, whereas human samples represent a multifactorial, chronic disease state shaped by genetic, environmental and microbial variables.[33] Additionally, differences in ILC subset definitions, such as reliance on surface markers (e.g., CRTH2 for human ILC2s) versus transcription factors in mice, may influence interpretations. For instance, human ILC3s identified as c-Kit+ cells might represent a heterogeneous population, potentially including precursors or transitional states not captured in mice.[34,35] These technical variations underscore the need for standardised ILC classification frameworks across species.

Several limitations temper the conclusions of this study. The small human sample limits statistical power and generalisability, particularly given the heterogeneity of periodontitis staging and patient demographics. Due to the small sample size, this study did not stratify by periodontitis substages. The exact relationship between ILC subset distribution and disease severity require further investigation in larger-scale studies. Ultimately, the proportional alterations in ILC subsets reported herein are founded on surface phenotyping. Direct functional validation via intracellular cytokine staining was hindered by substantial technical obstacles: the exceedingly low frequency of ILCs in gingival tissue (for instance, pooling bilateral gingiva from three mice yielded only approximately 100 ILCs) led

to cytokine signals falling below the reliable detection threshold of conventional flow cytometry. Future investigations utilising more sensitive single-cell technologies (such as single-cell RNA sequencing or high-parameter spectral flow cytometry) on larger pooled samples will be of critical significance for the definitive characterisation of the functional state and heterogeneity of periodontal ILCs. Moreover, the human samples were derived from severe periodontitis cases (Stage III/IV) and encompassed a broader age range than the young adult mice. Aging is known to alter immune cell function and the inflammatory microenvironment. Although this restricts direct mechanistic extrapolation, it emphasises the significance of our comparative approach in identifying both conserved and species-/age-specific ILC responses, laying a foundation for future studies using aged mouse models. Although our study offers a fundamental characterisation of ILC subsets during the active phase of disease pathogenesis, the function of these cells in the protracted chronic phase, which may entail persistent inflammation and tissue remodelling, necessitates further investigation. Single-cell transcriptomic profiling could resolve heterogeneity within ILC populations and identify novel biomarkers or therapeutic targets. In vitro co-culture experiments with periodontal pathogens (e.g., *Porphyromonas gingivalis*) may clarify how ILCs interact with microbial communities to shape immune responses. Additionally, interventional studies in murine models, such as IL-17/IL-22 blockade or adoptive transfer of specific ILC subsets, could establish causal relationships between ILC dynamics and bone loss. In human research, longitudinal studies tracking ILC changes across disease stages and in response to therapies would elucidate their clinical relevance. Finally, exploring crosstalk between ILCs and other immune cells, such as neutrophils, macrophages or Th17 cells, may uncover synergistic pathways driving inflammation.[36-38]

Conclusion

This work emphasises how ILCs are differentially related to periodontitis, which is a balanced, protective and harmful function in a context-dependent way. Although murine models point to ILC2s loss as a tipping point towards acute inflammation, ILC3s are found in human data as central orchestrators of chronic illness. These observations support the use of species-specific therapeutic approaches: increasing ILC2s activity to address inflammation in acute situations, as opposed to focusing on cytokines generated from ILC3s-derived cytokines (e.g., IL-17) in chronic human periodontitis. This work paves the way for novel immunomodulatory treatments that complement traditional antimicrobial approaches by deciphering the heterogeneity and plasticity of ILCs, which ultimately aims to restore immune homeostasis in periodontal tissues.

Conflicts of interest

The authors declare no conflicts of interest related to this study.

Author contribution

Drs Wen Min ZENG and Zong Xian LI contributed to the methodology, data acquisition, data analysis and drafting of the manuscript; Dr Yu Feng XIE contributed to the conceptualisation, methodology, investigation and supervision; Drs Chao ZHONG and Xiao Qian YU contributed to the conceptualisation, methodology, investigation, supervision, review and editing of the manuscript.

(Received Oct 01, 2025, accepted Mar 12, 2026)

References

1. Hajishengallis G. Periodontitis: From microbial immune subversion to systemic inflammation. Nat Rev Immunol 2015;15:30–44.
2. Kwon T, Lamster IB, Levin L. Current concepts in the management of periodontitis. Int Dent J 2021;71:462–476.
3. Kinane DF, Stathopoulou PG, Papapanou PN. Periodontal diseases. Nat Rev Dis Primers 2017;3:17038.
4. Artis D, Spits H. The biology of innate lymphoid cells. Nature 2015;517(7534):293–301.
5. Mjösberg J, Spits H. Human innate lymphoid cells. J Allergy Clin Immunol 2016;138:1265–1276.
6. Colonna M. Innate lymphoid cells: Diversity, plasticity, and unique functions in immunity. Immunity 2018;48:1104-1117.
7. Srivastava RK, Sapra L, Bhardwaj A, Mishra PK, Verma B, Baig Z. Unravelling the immunobiology of innate lymphoid cells (ILCs): Implications in health and disease. Cytokine Growth Factor Rev 2023;74:56–75.
8. Saez A, Gomez-Bris R, Herrero-Fernandez B, et al. Innate lymphoid cells in intestinal homeostasis and inflammatory bowel disease. Int J Mol Sci 2021;22:e13527.
9. Guia S, Narni-Mancinelli E. Helper-like innate lymphoid cells in humans and mice. Trends Immunol 2020;41:436–452.
10. Li C, Liu J, Pan J, Wang Y, Shen L, Xu Y. ILC1s and ILC3s exhibit inflammatory phenotype in periodontal ligament of periodontitis patients. Front Immunol 2021;12:708678.
11. Qin X, Hoda MN, Susin C, et al. Increased innate lymphoid cells in periodontal tissue of the murine model of periodontitis: The role of AMP-activated protein kinase and relevance for the human condition. Front Immunol 2017;8:922.
12. Kindstedt E, Koskinen Holm C, Palmqvist P, et al. Innate lymphoid cells are present in gingivitis and periodontitis. J Periodontol 2019;90:200–207.

13. Dutzan N, Konkel JE, Greenwell-Wild T, Moutsopoulos NM. Characterization of the human immune cell network at the gingival barrier. Mucosal Immunol 2016;9:1163–1172.
14. Zeng W, Liu G, Luan Q, et al. B-cell deficiency exacerbates inflammation and bone loss in ligature-induced experimental periodontitis in mice. J Inflamm Res 2021;14:5367–5380.
15. Virto L, Haugen HJ, Fernández-Mateos P, et al. Melatonin expression in periodontitis and obesity: An experimental in-vivo investigation. J Periodontal Res 2018;53:825–831.
16. Leupold T, Wirtz S. ILCs-Crucial players in enteric infectious diseases. Int J Mol Sci 2022;23:14200.
17. Kim HY, Umetsu DT, Dekruyff RH. Innate lymphoid cells in asthma: Will they take your breath away? Eur J Immunol 2016;46:795–806.
18. Moutsopoulos NM, Konkel JE. Tissue-Specific Immunity at the oral mucosal barrier. Trends Immunol 2018;39:276–287.
19. Schinnerling K, Penny HA, Soto JA, Melo-Gonzalez F. Immune responses at host barriers and their importance in systemic autoimmune diseases. Adv Exp Med Biol 2023;1408:3–24.
20. Seillet C, Belz GT, Huntington ND. Development, homeostasis, and heterogeneity of NK cells and ILC1. Curr Top Microbiol Immunol 2016;395:37–61.
21. Klose CSN, Flach M, Möhle L, et al. Differentiation of type 1 ILCs from a common progenitor to all helper-like innate lymphoid cell lineages. Cell 2014;157:340–356.
22. Kabata H, Moro K, Koyasu S. The group 2 innate lymphoid cell (ILC2) regulatory network and its underlying mechanisms. Immunol Rev 2018;286:37–52.
23. Melo-Gonzalez F, Hepworth MR. Functional and phenotypic heterogeneity of group 3 innate lymphoid cells. Immunology 2017;150:265–275.
24. Koprivica I, Stanisavljević S, Mićanović D, Jevtić B, Stojanović I, Miljković Đ. ILC3: A case of conflicted identity. Front Immunol 2023;14:1271699.
25. Hoffmann JP, Kolls JK, McCombs JE. Regulation and function of ILC3s in pulmonary infections. Front Immunol 2021;12:672523.
26. Slots J. Periodontitis: Facts, fallacies and the future. Periodontol 2000 2017;75:7–23.
27. Zeng B, Shi S, Ashworth G, Dong C, Liu J, Xing F. ILC3 function as a double-edged sword in inflammatory bowel diseases. Cell Death Dis 2019;10:315.
28. Abusleme L, Moutsopoulos NM. IL-17: Overview and role in oral immunity and microbiome. Oral Dis 2017;23:854–865.
29. Moutsopoulos NM, Lionakis MS, Hajishengallis G. Inborn errors in immunity: Unique natural models to dissect oral immunity. J Dent Res 2015;94:753–758.
30. Bunte K, Beikler T. Th17 cells and the IL-23/IL-17 axis in the pathogenesis of periodontitis and immune-mediated inflammatory diseases. Int J Mol Sci 2019;20:3394.
31. Neurath N, Kesting M. Cytokines in gingivitis and periodontitis: From pathogenesis to therapeutic targets. Front Immunol 2024;15:1435054.
32. Dutzan N, Kajikawa T, Abusleme L, et al. A dysbiotic microbiome triggers T(H)17 cells to mediate oral mucosal immunopathology in mice and humans. Sci Transl Med 2018;10:eaat0797.
33. Lin P, Niimi H, Ohsugi Y, et al. Application of ligature-induced periodontitis in mice to explore the molecular mechanism of periodontal disease. Int J Mol Sci 2021;22:8900.
34. Vacca P, Chiossone L, Mingari MC, Moretta L. Heterogeneity of NK cells and other innate lymphoid cells in human and murine decidua. Front Immunol 2019;10:170.
35. Montaldo E, Juelke K, Romagnani C. Group 3 innate lymphoid cells (ILC3s): Origin, differentiation, and plasticity in humans and mice. Eur J Immunol 2015;45:2171–2182.
36. Yin G, Zhao C, Pei W. Crosstalk between macrophages and innate lymphoid cells (ILCs) in diseases. Int Immunopharmacol 2022;110:108937.
37. Sonnenberg GF, Hepworth MR. Functional interactions between innate lymphoid cells and adaptive immunity. Nat Rev Immunol 2019;19:599–613.
38. Withers DR. Innate lymphoid cell regulation of adaptive immunity. Immunology 2016;149:123–130.