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Expression of interleukin-33 and its receptor ST2 in periapical granulomas and radicular cysts

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BACKGROUND: Interleukin-33 (IL-33) is a recently identified cytokine belonging to the IL-1 family and ligand for the IL-1 receptor-related protein ST2. IL-33/ST2 signaling plays a critical role in allergy, autoimmunity, and chronic inflammatory disorders, but its role in the pathogenesis of periapical lesions is unknown. We aimed to investigate the expression patterns of IL-33 and ST2 in human periapical lesions.

METHODS: Periapical lesions (n = 36) and healthy periapical tissues (n = 10) were evaluated by immunohistochemistry using antibodies specific for human IL-33 and ST2. Lesion samples were further analyzed by double immunofluorescence to assess IL-33/ST2 co-expression.

RESULTS: The numbers of IL-33- and ST2-positive fibroblasts were significantly higher in periapical lesions compared to healthy periapical tissues (both $P < 0.05$), while the numbers of IL-33- and ST2-positive endothelial cells were similar (both $P > 0.05$). There were no significant differences in the numbers of IL-33- and ST2-positive fibroblasts and endothelial cells between periapical granulomas and radicular cysts (all $P > 0.05$). Similarly, numbers of ST2-positive mononuclear cells did not differ between periapical granulomas and radicular cysts ($P > 0.05$). The majority of epithelial cells in radicular cysts were IL-33 positive, while the small proportion of epithelial cells was ST2 positive. Double immunofluorescence analysis revealed IL-33/ST2 co-expression in fibroblasts and endothelial cells.

CONCLUSIONS: IL-33 and ST2 are expressed in periapical granulomas and radicular cysts. Increased numbers of IL-33- and ST2-positive fibroblasts in periapical lesions when compared to healthy periapical tissues suggest that IL-33/ST2 signaling may be involved in periapical inflammation and tissue fibrosis.

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Introduction

Periapical lesions are a common pathology within the human population which develops in response of periapical tissue to chronic stimulation caused by microorganisms that invade and destroy the dental pulp (1). Persistence of inflammatory stimuli is associated with resorption of surrounding bone, which is replaced by granulation tissue infiltrated by macrophages, lymphocytes, and plasma cells to form a periapical granuloma (2–5). A sequel to granuloma formation is the proliferation of the Malassez epithelium associated with the inflammation, which may lead to the development of radicular cyst (2, 5).

Cytokines are involved in the pathogenesis of periapical lesions; it has been reported that T helper 1 (Th1) and Th17 cytokines together with IL-1, IL-6 and tumor necrosis factor (TNF)-α promote inflammation, bone resorption, and disease progression, whereas Th2 and T regulatory cell (Treg)-derived cytokines are of importance in healing processes occurring in the advanced stage of lesion development characterized by downregulation of the inflammatory response (6–8). However, the regulation of the cytokine network during the development and maintenance of chronic inflammation in periapical lesions is not elucidated.

IL-33 is the most recent addition to the IL-1 cytokine family, which also includes IL-1α, IL-1β, IL1Ra (IL-1 receptor antagonist), and IL-18. Like IL-1α, IL-33 is a dual-function cytokine that may act as both an intracellular nuclear factor and an extracellular cytokine (9, 10). IL-33 is mainly expressed by fibroblasts, and endothelial and epithelial cells (10–12). In addition, IL-33 expression can be induced in both resident and infiltrating cells in inflamed tissues (13–16). IL-33 is specifically released as an 'endogenous' danger signal or 'alarmin' by damaged, stressed, or necrotic cells to alert the immune system of a local threat

(11). Extracellular effects of IL-33 are mediated by binding to its receptor ST2, in association with IL-1R accessory protein (IL-1RacP), leading to the activation of nuclear factor-kB (NF-kB) and mitogen-activated protein (MAP) kinases. In contrast to other members of IL-1 family, which predominantly promote Th1 type responses, IL-33 mainly induces production of Th2 cytokines (9).

ST2 is a member of the IL-1 receptor family that exists in two forms: a transmembrane full length form (ST2L) and a shorter soluble secreted form (sST2) due to differential mRNA processing within the ST2 gene (17). ST2L is an orphan receptor expressed by many hematopoietic cells, granulocytes, macrophages, dendritic cells, mast cells, and NK and NKT cells and is selectively expressed by Th2 cells (18). Soluble sST2 acts as a decoy receptor for IL-33 (19).

IL-33/ST2 signaling has a dual role in different chronic inflammatory disorders exhibiting a protective role in Th1/Th17 cell-mediated inflammatory diseases, such as experimental autoimmune encephalomyelitis (20), fulminant hepatitis (21), and type 1 diabetes (22), and enhances inflammation in asthma (23) and antigen-induced arthritis (24). The data regarding IL-33/ST2 expression in periapical lesions are very scarce. Although the expression of IL-33 has recently been demonstrated at the level of mRNA in human periapical granulomas (25), the expression of its receptor as well as the cellular sources of IL-33 and ST2 in the periapical lesions is unknown. Therefore, we aimed to investigate IL-33/ST2 expression patterns in patients with chronic apical periodontitis.

Materials and methods

Preparation of samples

The study was approved by the Ethics Committee of the Faculty of Stomatology, University of Belgrade, Serbia, and Ethics Committee of the Faculty of Medical Sciences, University of Kragujevac, Serbia, in accordance with provisions of the Declaration of Helsinki. Written and informed consents were obtained from all patients prior to the collection of samples.

The diseased tissue samples comprised periapical lesions (n = 36) obtained from patients who had been diagnosed as having apical periodontitis based on clinical manifestations (the absence of pulpal responses by an electric pulp tester, the absence of throbbing pain, the presence of pain on pulpation of the mucosa around the tooth apex and percussion pain) (26) and radiographic evidence of periapical alveolar bone loss. Periapical lesions were collected from patients at the time of dental extraction or apical surgery. The mean age of patients (24 men and 22 women) was 44.28 ± 1.98 years, with a range from 19 to 64 years. Tooth removal was indicated when apicoectomy deemed impossible to perform (teeth with inadequate periodontal support and excessive mobility, lack of motivation for apical surgery in patients who previously experienced endodontic treatment, as well as financial constraints). The teeth of 21 patients were endodontically treated.

The healthy tissue control comprised samples of periodontal ligaments (n = 10) obtained from the middle third of the dental root of healthy permanent premolars extracted for orthodontic purposes. The mean age of patients (three men and seven women) was 22.1 ± 0.79 years, with a range from 18 to 25 years. All patients were without systemic diseases and were not under antibiotics for the least 1 month.

Tissue samples were immediately placed into transport medium consisting of RPMI-1640 medium (Sigma, Munich, Germany) and antibiotics (60 μg/ml of gentamycin; ICN, Costa Mesa, CA, USA; 100 U/ml of penicillin and 0.1 mg/ml streptomycin; Galenika, Belgrade, Serbia)/antimycotics (100 μg/ml of amphotericin; Sigma) and transported to laboratory. Tissues were frozen and kept at −70°C, until cryosections (4 μm thickness) were prepared for immunohistological analyses.

Histological examination

Two periapical cryosections per tissue sample were used for histological examination, which was based on hematoxylin and eosin (H&E) staining. An experienced pathologist analyzed the samples using light microscope (Carl Zeiss: Axioscop 40, Norway). Periapical lesions were classified as granulomas and cysts, according to the histological characteristics.

Immunohistochemical analysis

Immunohistochemistry was performed to identify IL-33 and ST2 expression in periapical tissues. Tissue cryosections were incubated with primary biotinylated mouse monoclonal anti-IL-33 antibody (ab54384, Abcam, Cambridge, UK; dilution 1:100) and primary rabbit polyclonal anti-ST2 antibody (PA5-20077; Thermo Scientific, Waltham, MA, USA; dilution 1:500) and visualized using LP Detection System (TL-015-HD, Thermo Scientific, USA). Sections were counterstained with hematoxylin, photomicrographed with a digital camera (Canon PC 1089, USA) mounted on light microscope, digitized, and analyzed. Brown colored cells were considered as positive.

Negative controls consisted of sections in which primary antibodies were pre-incubated with blocking peptides (human IL-33 protein fragment, ab82840, Abcam, UK and ST2 synthetic peptide, PEP-0195, Thermo Scientific) or were omitted and replaced with either mouse IgG1 biotin isotype control (ab37358, Abcam) or rabbit IgG (ab27472). Positive controls consisted of IL-33 and ST2 staining on healthy human skin sections.

Morphometric analysis

Morphometric analysis consisted of the quantification of the number of positive cells using images of the histological sections captured with a digital system and analyzed with the software ImageJ 1.36 (National Institutes of Health, Bethesda, MD, USA), as previously described (27). The specimens were scored by counting cells over five different fields of vision in one section. Each field to be quantified was captured with a camera and the number of cells in each field was determined, as well as the area of each field. The density of positive cells was expressed as the number of cells per square millimeter (cells/mm^2). The average number of positive cells was calculated for each specimen.

Double immunofluorescence analysis

Double immunofluorescent staining was performed to investigate whether IL-33 and its receptor ST2 are co-expressed in the periapical lesions. Immunofluorescence of periapical lesion cryosections was performed using rabbit polyclonal anti-ST2 antibody (PA5-20077; Thermo Scientific; dilution 1:500), followed by incubation with FITC-conjugated donkey anti-rabbit antibody (ab98492, Abcam;1:250), followed by biotinylated mouse monoclonal anti-IL-33 antibody (ab54384, Abcam; 1:100) and streptavidin PE (1:1000). The sections were mounted with Pro Long Gold antifade reagent with DAPI and analyzed using ZEISS AXIOVERT Microscope (Germany).

Statistical analysis

All statistics were carried out using statistical package SPSS, version 13 (North Castle, NY, USA). Descriptive statistics including the mean and standard error (mean ± SEM) were calculated. Comparisons between groups were performed using Mann–Whitney *U*-test. The results were considered significantly different when $P < 0.05$.

Results

Histological evaluation of periapical lesions

Analysis of hematoxylin-stained sections using light microscopy demonstrated a varied histology of periapical lesions. Twenty specimens exhibited granulomatous lesions with infiltration of inflammatory cells. Epithelium was absent in these samples, and the specimens were considered as periapical granulomas. Other 16 lesions exhibited cavities delimited by epithelium and fibrous connective tissue walls. These specimens were therefore considered as radicular cysts (data not shown).

Immunohistochemical analysis

To identify IL-33- and ST2-expressing cells in periapical tissues, immunohistochemical staining using monoclonal anti-IL-33 and polyclonal anti-ST2 antibodies was performed. IL-33 expression was observed in the nuclei of cells morphologically consistent with fibroblasts and endothelial cells in periapical lesions (Fig. 1A, left and right) and healthy periapical tissues (Fig. 1B, left and right). Contrary to IL-33 staining in all endothelial cells (Fig. 1A and B,

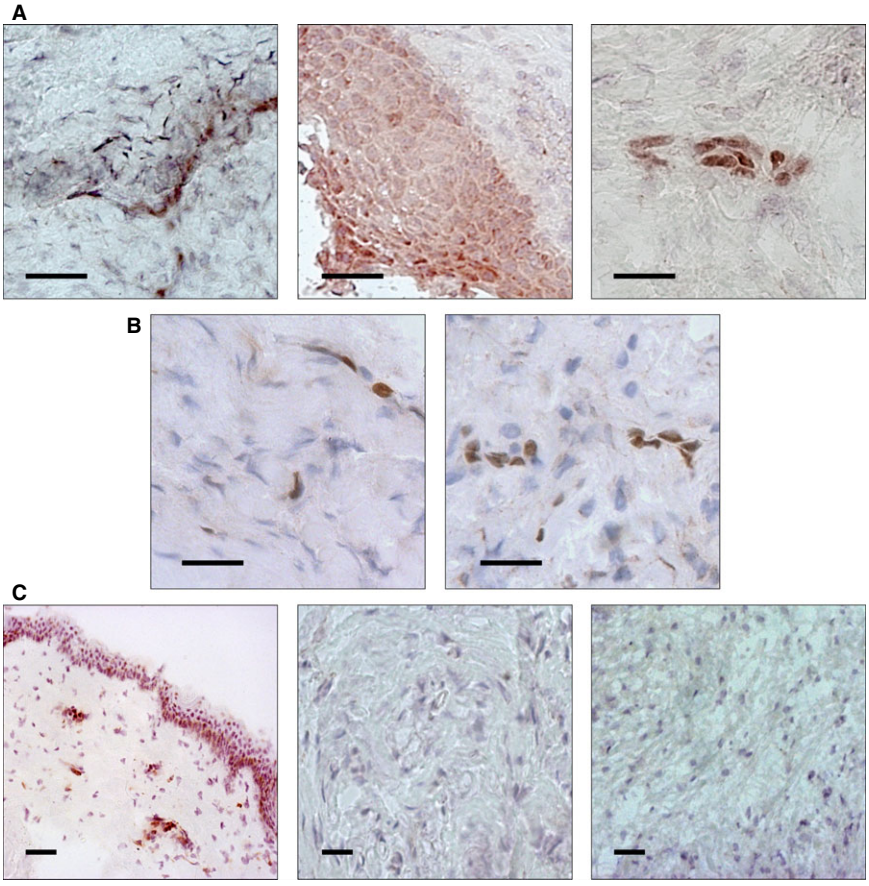

Figure 1 Photomicrographs of representative IL-33-positive staining in periapical tissues. (A) In periapical lesions, IL-33 staining was observed in the cells morphologically consistent with fibroblasts (left), endothelial cells (right) and epithelial cells (medium). Original magnification, 400×; Scale bar = 50 μm. (B) In healthy periapical tissues, IL-33 staining was observed in the cells morphologically consistent with fibroblasts (left) and endothelial cells (right). Original magnification, 400×; Scale bar = 50 μm. (C) Positive control – healthy human skin (left) demonstrating IL-33 expression in keratinocytes and dermal endothelial cells. Original magnification, 100×; Scale bar = 100 μm; negative controls did not show any positive staining: Immunohistochemical analyses of periapical tissues using monoclonal anti-IL-33 antibody pre-incubated with blocking peptide (medium) and isotype control antibody (right). Original magnification, 200×; Scale bar = 50 μm.

right), IL-33-positive fibroblasts were almost absent in healthy periapical tissues (Fig. 1B, left). In radicular cysts, IL-33 was also expressed in the cytoplasm and nucleus of all epithelial cells (Fig. 1A, middle). This staining was specific as it was observed with monoclonal anti-IL-33 antibody (Fig. 1A,B) and it was abrogated by pre-incubating the antibody with human IL-33 protein fragment (Fig. 1C, medium) or using isotype control antibody (Fig. 1C, right). In healthy human skin, IL-33 expression was clearly demonstrated in keratinocytes and dermal endothelial cells (Fig. 1C, left).

The cellular expression of ST2 receptor was similar, with additional staining of mononuclear cells morphologically consistent with lymphocytes or macrophages in periapical lesions (Fig. 2A, lower right). ST2 staining was specific as it was observed with polyclonal anti-ST2 antibody (Fig. 2A,B) and it was abrogated by pre-incubating the antibody with ST2 blocking peptide (Fig. 2C, medium) or using isotype control antibody (Fig. 2C, right). In healthy human skin, ST2 expression was demonstrated in keratinocytes, dermal endothelial cells, and fibroblasts (Fig. 2C, left).

Next, the number of IL-33- and ST2-immunostained cells in the periapical tissues was determined (Table 1A and B). When statistical difference was calculated using Mann–Whitney U-test, the numbers of IL-33-positive fibroblasts/mm^2 were significantly higher in both periapical granulomas and radicular cysts when compared to healthy periapical tissues (both $P < 0.001$). Similar numbers of IL-33-positive endothelial cells were observed in periapical lesions and healthy tissue controls ($P > 0.05$). There were no significant differences in the numbers of IL-33-positive fibroblasts and endothelial cells between periapical granulomas and radicular cysts (both $P > 0.05$). However, it should be noted that in radicular cysts, epithelial cells were prevalent IL-33-positive cells.

The number of ST2-positive fibroblasts was significantly higher in both periapical granulomas and radicular cysts when compared to healthy periapical tissues ($P < 0.001$ and $P = 0.005$, respectively). Similar quantities of ST2-positive endothelial cells were observed in periapical lesions and healthy tissue controls ($P > 0.05$). Further, there were no significant differences in the numbers of ST2-positive fibroblasts, and endothelial and mononuclear cells between periapical granulomas and radicular cysts (all $P > 0.05$). Most epithelial cells were ST2 negative in radicular cysts.

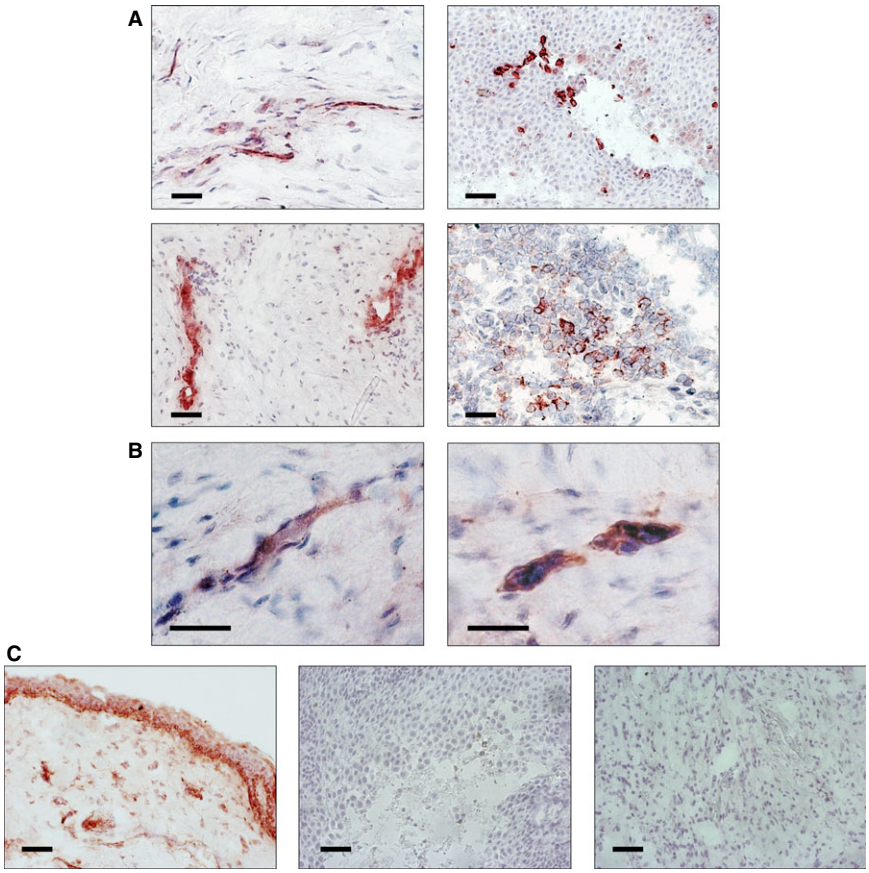

Figure 2 Photomicrographs of representative ST2-positive staining in periapical tissues. (A) In periapical lesions, ST2 staining was observed in the cells morphologically consistent with fibroblasts (upper left), endothelial (lower left), epithelial (upper right), and mononuclear cells (lower right). Original magnification, 200×; Scale bar = 50 μm. (B) In healthy periapical tissues, ST2 staining was observed in the cells morphologically consistent with fibroblasts (left) and endothelial cells (right). Original magnification, 400×; Scale bar = 50 μm. (C) Positive control – healthy human skin (left) demonstrating ST2 expression in keratinocytes, dermal endothelial cells, and fibroblasts. Original magnification, 100×; Scale bar = 100 μm; negative controls did not show any positive staining: Immunohistochemical analyses of periapical tissues using anti-ST2 antibody pre-incubated with blocking peptide (medium) and control antibody (right). Original magnification, 200×; Scale bar = 50 μm.

Table 1 IL-33 and ST2 expression in periapical tissues

A. IL-33

Tissue type	n	*Fibroblasts*	*Endothelial cells*	*Epithelial cells*
Healthy periapical tissue	10	5.83 ± 0.8	9.95 ± 1.24	–
Periapical granuloma	20	14.38 ± 1.15*	9.41 ± 0.91	–
Radicular cyst	16	12.63 ± 0.82*	8.81 ± 0.58	47.39 ± 2.63

B. ST2

Tissue type	n	*Fibroblasts*	*Endothelial cells*	*MNCs*	*Epithelial cells*
Healthy periapical tissue	10	10.2 ± 0.62	7.69 ± 0.60	–	–
Periapical granuloma	20	13.16 ± 0.79*	8.25 ± 0.81	15.30 ± 1.13	–
Radicular cyst	16	12.45 ± 1.04^{+}	7.89 ± 0.78	16.71 ± 1.81	13.97 ± 1.45

Statistically significant difference compared with healthy periapical tissue in the IL-33 or ST2 stained group: *$P < 0.001$; $^{+}P = 0.005$.
Data are presented as numbers of IL-33- or ST2-positive cells (mean ± SEM) per mm² of periapical tissues.

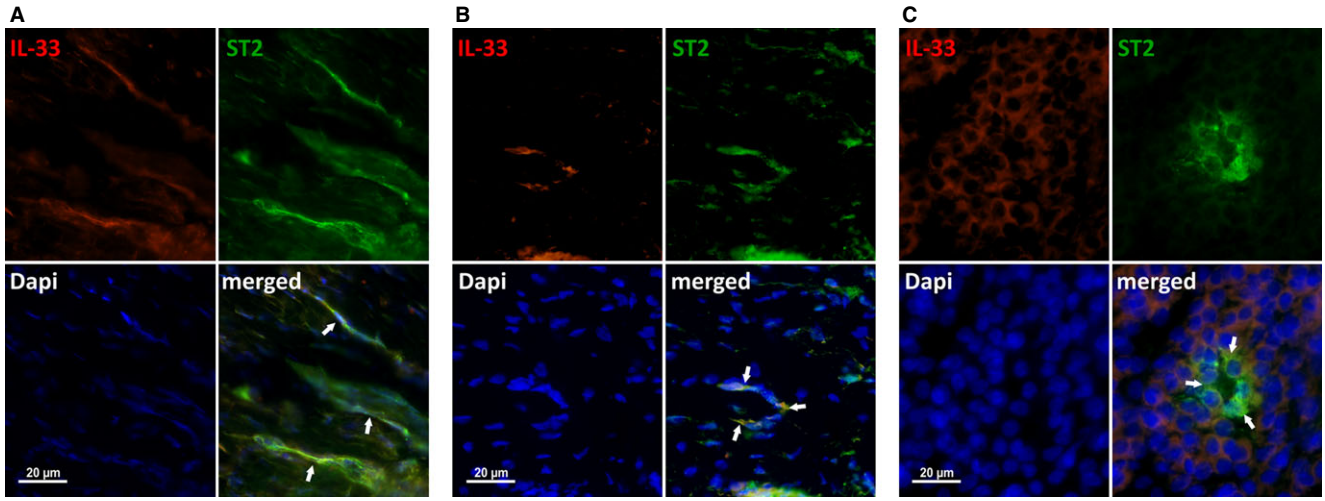

Figure 3 Representative images of periapical lesions double stained with monoclonal antibody for IL-33 and polyclonal antibody for ST2. IL-33 and its receptor ST2 were co-expressed in the cells morphologically consistent with fibroblasts (A) and endothelial cells (B) (arrows show double expression). Mononuclear cells were ST2 single positive (arrows) (C).

Double immunofluorescence analysis

Double immunofluorescent staining was performed to assess the co-expression of IL-33 and its receptor ST2 in periapical lesions. Most IL-33 strongly/moderately positive cells morphologically consistent with fibroblasts were strongly ST2 positive (Fig. 3A). Further, most IL-33 moderately/weakly positive cells morphologically consistent with endothelial cells were strongly/moderately ST2 positive (Fig. 3B). However, most ST2 strongly/moderately positive mononuclear cells morphologically consistent with lymphocytes or macrophages were IL-33 negative (Fig. 3C). Therefore, IL-33/ST2 co-expression was detected in the cells morphologically consistent with fibroblasts and endothelial cells, whereas lymphocyte-like or macrophage-like cells expressed only IL-33 receptor (ST2).

Discussion

In the present study, we investigated the expression of IL-33 and its receptor, ST2 in human periapical lesions. We are the first to demonstrate that ST2 is expressed in the periapical tissues. Immunohistochemical analysis showed similar expression patterns of IL-33 and ST2 in periapical granulomas and radicular cysts. These findings suggest that the IL-33/ST2 axis is activated in periapical lesions, so we performed double immunofluorescence analysis to examine IL-33/ST2 co-expression.

Up to now, the expression of IL-33 has been studied only at the level of mRNA in periapical granulomas (25), although few reports concerning IL-33 in periodontal disease were published. It has been found that gingival crevicular fluid concentrations of IL-33 are significantly higher in chronic periodontitis individuals compared with healthy individuals (28). Recently, it has been also shown that TNF-α induces IL-33 expression in human gingival fibroblasts isolated from human periodontitis samples (29). In addition, increased expression of IL-33 has been demonstrated in an experimental rat model of periodontitis (30). To better understand the role of IL-33 in chronic apical periodontitis, we investigated the expression pattern of IL-33 in both periapical granulomas and radicular cysts, as well as in healthy periapical tissues. We demonstrated the

expression of IL-33 in the nucleus of fibroblasts and endothelial cells in periapical lesions and periodontal ligaments, and in the nucleus and cytoplasm of epithelial cells in radicular cysts (Fig. 1). These observations are in agreement with previous findings about the cellular sources of IL-33 (10–12). Surprisingly, although IL-33 was generally not detected in fibroblasts from normal human tissues except in lymphoid tissue (31), we found IL-33 expression in these cells from control periodontal ligaments. Expression of IL-33 in all endothelial and epithelial cells is in agreement with the report of Moussion et al. (11) that IL-33 is constitutively expressed in these cells.

Morphometric analysis showed significant increase of IL-33-positive fibroblasts in periapical lesions (Fig. 1A, left) in comparison with healthy periapical tissues (Fig. 1B, left). These results could be explained by the fact that pro-inflammatory stimuli induce expression of IL-33 in fibroblasts (15, 16), and suggest possible implication of IL-33 in the process of wound healing and tissue fibrosis in periapical lesions (32, 33). Nuclear staining of endothelium in our tissue samples is in line with reports of IL-33 staining in normal and chronically inflamed human tissues, such as human skin, small intestine, lung (34), chronically inflamed rheumatoid arthritis synovium, and Crohn's disease intestine (10). Taken together, these findings provide further support to the possibility that IL-33 is a chromatin-associated nuclear factor of endothelial cells (10). Interestingly, we found both nuclear and cytoplasmic expression of IL-33 in epithelial cells in radicular cysts (Fig. 1A, medium). IL-33 in the cytoplasm of epithelial cells could be related to the release of IL-33 from the nucleus to exert its function as an endogenous 'danger' signal or 'alarmin' (11).

It is well known that IL-33 affects the function of cells that express ST2 molecule (9). The ST2 gene was originally identified in fibroblasts (35, 36). Recently, ST2 mRNA expression was demonstrated in endothelial and epithelial cells (37). Consistent with these findings, we observed ST2 expression in fibroblasts and endothelial cells in periapical lesions (Fig. 2A, upper and lower left) and healthy periapical tissues (Fig. 2B, left and right), as well as in the epithelium of radicular cysts (Fig. 2A, upper right). We have noticed that not every epithelial cell was ST2 positive. It is not surprising, as it was shown that Th2 cytokines upregulate ST2 gene expression in these cells (37), and both Th1 and Th2 immune responses are involved in the pathogenesis of radicular cysts (38). Further, we observed ST2 staining of mononuclear cells in periapical lesions (Fig. 2A, lower right). De Paula-Silva et al. (39) have shown that mononuclear cells are the most prevalent cells in both granulomas and cysts. Morphometric analysis demonstrated that the numbers of ST2-positive fibroblasts were significantly higher in periapical lesions compared to healthy periapical tissues. In line with these results, Tajima et al. (40) showed that ST2 gene expression induced by Th2 and proinflammatory cytokines in lung fibrosis model and human lung fibroblast cell line, possibly reflecting the development of both the inflammatory and the fibrotic processes. Data presented here are in the apparent contrast with previous finding that ST2L expression is absent in the periapical lesions cells obtained by tissue digestion and analyzed by flow cytometry (41).

Double immunofluorescence was performed to analyze whether IL-33 and ST2 are co-expressed in the periapical lesions. IL-33/ST2 co-expression has been recently shown in decidual stromal cells in human first-trimester pregnancy (42). We found IL-33/ST2 co-expression in fibroblasts and endothelial cells (Fig. 3). Therefore, it is conceivable to assume that IL-33 could exert autocrine effects by binding to ST2 in these cells. IL-33/ST2 signaling has dual, pro-inflammatory, or protective roles in different inflammatory diseases depending on the immune mechanisms underlying the pathogenesis of each disease condition (20–24). The data on the role of IL-33/ST2 signaling in the pathogenesis of periapical lesions are lacking. Our recent results show that IL-33/ST2 pathway negatively regulates inflammatory bone destruction in experimentally induced periapical lesions in mice by preventing Th1/Th17 cell-mediated immune responses (43). Therefore, IL-33/ST2 signaling might be important for the restriction of proinflammatory immune response in the periapical lesions, which remains to be further elucidated.

Conclusions

IL-33 and its receptor ST2 are expressed in human periapical granulomas and radicular cysts. IL-33/ST2 signaling might have important role in the pathogenesis of chronic apical periodontitis in humans and could represent the promising targets for therapeutic intervention.

References

1. Nair PN. Pathogenesis of apical periodontitis and the causes of endodontic failures. *Crit Rev Oral Biol Med* 2004; **15**: 348–81.
2. Gao Z, Mackenzie IC, Rittman BR, et al. Immunocytochemical examination of immune cells in periapical granulomata and odontogenic cysts. *J Oral Pathol* 1988; **17**: 84–90.
3. Lukic A, Arsenijevic N, Vujanic G, Ramic Z. Quantitative analysis of the immunocompetent cells in periapical granuloma: correlation with the histological characteristics of the lesions. *J Endod* 1990; **16**: 119–22.
4. Marton IJ, Kiss C. Protective and destructive immune reactions in apical periodontitis. *Oral Microbiol Immunol* 2000; **15**: 139–50.
5. Liapatas S, Nakou M, Rontogianni D. Inflammatory infiltrate of chronic periradicular lesions: an immunohistochemical study. *Int Endod J* 2003; **36**: 464–71.
6. Kawashima N, Stashenko P. Expression of bone-resorptive and regulatory cytokines in murine periapical inflammation. *Arch Oral Biol* 1999; **44**: 55–66.
7. Colic M, Vasilijic S, Gazivoda D, Vucevic D, Marjanovic M, Lukic A. Interleukin-17 plays a role in exacerbation of inflammation within chronic periapical lesions. *Eur J Oral Sci* 2007; **115**: 315–20.
8. Colic M, Gazivoda D, Vucevic D, Vasilijic S, Rudolf R, Lukic A. Proinflammatory and immunoregulatory mechanisms in periapical lesions. *Mol Immunol* 2009; **47**: 101–13.
9. Schmitz J, Owyang A, Oldham E, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* 2005; **23**: 479–90.
10. Carriere V, Roussel L, Ortega N, et al. IL-33, the IL-1-like cytokine ligand for ST2 receptor, is a chromatin-associated

nuclear factor in vivo. *Proc Natl Acad Sci USA* 2007; **104**: 282–7.
11. Moussion C, Ortega N, Girard JP. The IL-1-like cytokine IL-33 is constitutively expressed in the nucleus of endothelial cells and epithelial cells *in vivo*: a novel 'alarmin'? *PLoS ONE* 2008; **3**: e3331.
12. Sanada S, Hakuno D, Higgins L, et al. IL-33 and ST2 comprise a critical biomechanically induced and cardioprotective signaling system. *J Clin Invest* 2007; **117**: 1538–49.
13. Prefontaine D, Lajoie-Kadoch S, Foley S, et al. Increased expression of IL-33 in severe asthma: evidence of expression by airway smooth muscle cells. *J Immunol* 2009; **183**: 5094–103.
14. Kobori A, Yagi Y, Imaeda H, et al. Interleukin-33 expression is specifically enhanced in inflamed mucosa of ulcerative colitis. *J Gastroenterol* 2010; **45**: 999–1007.
15. Palmer G, Talabot-Ayer D, Lamacchia C, et al. Inhibition of interleukin-33 signaling attenuates the severity of experimental arthritis. *Arthritis Rheum* 2009; **60**: 738–49.
16. Sponheim J, Pollheimer J, Olsen T, et al. Inflammatory bowel disease-associated interleukin-33 is preferentially expressed in ulceration-associated myofibroblasts. *Am J Pathol* 2010; **177**: 2804–15.
17. Bergers G, Reikerstorfer A, Braselmann S, et al. Alternative promoter usage of the Fos-responsive gene Fit-1 generates mRNA isoforms coding for either secreted or membrane-bound proteins related to the IL-1 receptor. *EMBO J* 1994; **13**: 1176–88.
18. Xu D, Chan WL, Leung BP, et al. Selective expression of a stable cell surface molecule on type 2 but not type 1 helper T cells. *J Exp Med* 1998; **187**: 787–94.
19. Hayakawa H, Hayakawa M, Kume A, Tominaga S. Soluble ST2 blocks interleukin-33 signaling in allergic airway inflammation. *J Biol Chem* 2007; **282**: 26369–80.
20. Milovanovic M, Volarevic V, Ljujic B, et al. Deletion of IL-33R (ST2) abrogates resistance to EAE in BALB/c mice by enhancing polarization of APC to inflammatory phenotype. *PLoS ONE* 2012; **7**: e45225.
21. Volarevic V, Mitrovic M, Milovanovic M, et al. Protective role of IL-33/ST2 axis in Con A-induced hepatitis. *J Hepatol* 2012; **56**: 26–33.
22. Zdravkovic N, Shahin A, Arsenijevic N, et al. Regulatory T cells and ST2 signaling control diabetes induction with multiple low doses of streptozotocin. *Mol Immunol* 2009; **47**: 28–36.
23. He X, Wu W, Lu Y, et al. Effect of interleukin-33 on Th1/Th2 cytokine ratio in peripheral lymphocytes in asthmatic mice. *Chin Med J* 2014; **127**: 1517–22.
24. Xu D, Jiang HR, Kewin P, et al. IL-33 exacerbates antigen-induced arthritis by activating mast cells. *Proc Natl Acad Sci USA* 2008; **105**: 10913–18.
25. Araujo-Pires AC, Francisconi CF, Biguetti CC, et al. Simultaneous analysis of T helper subsets (Th1, Th2, Th9, Th17, Th22, Tfh, Tr1 and Tregs) markers expression in periapical lesions reveals multiple cytokine clusters accountable for lesions activity and inactivity status. *J Appl Oral Sci* 2014; **22**: 336–46.
26. Lin LM, Huang GTJ. Pathobiology of the periapex. In: Hargreaves K, Cohen S, eds. *Pathways of the pulp*, 10th edn. Mosby: Elsevier, 2011; 529–58.
27. Marcal JR, Samuel RO, Fernandes D, et al. T-helper cell type 17/regulatory T-cell immunoregulatory balance in human radicular cysts and periapical granulomas. *J Endod* 2010; **36**: 995–9.
28. Buduneli N, Ozcaka O, Nalbantsoy A. Interleukin-33 levels in gingival crevicular fluid, saliva, or plasma do not differentiate chronic periodontitis. *J Periodontol* 2012; **83**: 362–8.
29. Beklen A, Tsaosus Memet G. Interleukin-1 superfamily member, interleukin-33, in periodontal disease. *Biotech Histochem* 2014; **89**: 209–14.
30. Köseoğlu S, Hatipoğlu M, Sağlam M, et al. Interlukin-33 could play an important role in the pathogenesis of periodontitis. *J Periodontol Res* 2014; doi: 10.1111/jre.12235 [Epub ahead of print].
31. Link A, Vogt TK, Favre S, Britschgi MR, et al. Fibroblastic reticular cells in lymph nodes regulate the homeostasis of naive T cells. *Nat Immunol* 2007; **8**: 1255–65.
32. Garlet GP, Horwat R, Ray HL Jr, et al. Expression analysis of wound healing genes in human periapical granulomas of progressive and stable nature. *J Endod* 2012; **38**: 185–90.
33. Tschumperlin DJ. Fibroblasts and the ground they walk on. *Physiology* 2013; **28**: 380–90.
34. Küchler AM, Pollheimer J, Balogh J, et al. Nuclear interleukin-33 is generally expressed in resting endothelium but rapidly lost upon angiogenic or proinflammatory activation. *Am J Pathol* 2008; **173**: 1229–42.
35. Tominaga S. A putative protein of a growth specific cDNA from BALB/c-3T3 cells is highly similar to the extracellular portion of mouse interleukin 1 receptor. *FEBS Lett* 1989; **258**: 301–4.
36. Klemenz R, Hoffmann S, Werenskiold AK. Serum- and oncoprotein-mediated induction of a gene with sequence similarity to the gene encoding carcinoembryonic antigen. *Proc Natl Acad Sci USA* 1989; **86**: 5708–12.
37. Yagami A, Kanami O, Morita H, et al. IL-33 mediates inflammatory responses in human lung tissue cells. *J Immunol* 2010; **185**: 5743–50.
38. Teixeira-Salum TB, Rodrigues DB, Gervasio AM, et al. Distinct Th1, Th2 and Treg cytokines balance in chronic periapical granulomas and radicular cysts. *J Oral Pathol Med* 2010; **39**: 250–6.
39. de Paula-Silva FW, D'Silva NJ, da Silva LA, Kapila YL. High matrix metalloproteinase activity is a hallmark of periapical granulomas. *J Endod* 2009; **35**: 1234–42.
40. Tajima S, Bando M, Ohno S, et al. ST2 gene induced by type 2 helper T cell (Th2) and proinflammatory cytokine stimuli may modulate lung injury and fibrosis. *Exp Lung Res* 2007; **33**: 81–97.
41. Colic M, Lukic A, Vucevic D, et al. Correlation between phenotypic characteristics of mononuclear cells isolated from human periapical lesions and their in vitro production of Th1 and Th2 cytokines. *Arch Oral Biol* 2006; **51**: 1120–30.
42. Hu WT, Li MQ, Liu W, et al. IL-33 enhances proliferation and invasiveness of decidual stromal cells by up-regulation of CCL2/CCR2 via NF-κB and ERK1/2 signaling. *Mol Hum Reprod* 2014; **20**: 358–72.
43. Velickovic M, Pejnovic N, Mitrovic S, Radosavljevic G, Jovanovic I, Kanjevac T, Jovicic N, Lukic A. ST2 Deletion Increases Inflammatory Bone Destruction in Experimentally Induced Periapical Lesions in Mice. *J Endod* 2015 Jan 13. doi: 10.1016/j.joen.2014.11.017. [Epub ahead of print]

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Conflict of interest

The authors declare no conflict of interest.