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Immunohistochemical analysis of apoptosis-related factors in lining epithelium of radicular cysts

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BACKGROUND: Some studies suggest that apoptosis-related factors are involved in the inflammatory processes of marginal periodontal lesions. However, the role of apoptosis in periapical inflammatory lesions remains unclear. We investigated the possible role of apoptotic cell death in periapical inflammatory lesions by means of immunohistochemical analysis of apoptosis-related factors and use of a cell proliferation marker.

METHODS: Paraffin-embedded sections of 19 radicular cysts (RCs), and five residual radicular cysts (RRCs) and control specimens of normal gingivae excised from seven cadavers were prepared and examined immunohistochemically with the use of monoclonal antibodies or polyclonal antisera against single-stranded DNA (ssDNA), p53, Bax, Bcl-2, caspase-3, Fas, Fas ligand (Fas-L), and Ki-67 antigen.

RESULTS: Epithelium of gingiva, RCs, and RRCs showed expression of ssDNA in suprabasal and superficial epithelial cells and Ki-67 reactivity in basal and parabasal cells. Expression of Ki-67 and ssDNA in RCs and RRCs was slightly higher than that in gingiva. Both Ki-67 and ssDNA reactivity in RCs with intense inflammatory reactions or with thick lining epithelium were significantly stronger than those in RCs with less inflammatory reactions or with thin lining epithelium. Reactivity for p53 was noted sporadically in epithelium of gingiva, RCs, and RRCs, and p53 expression in RCs was significantly greater than that in gingiva. Ki-67 and ssDNA reactivity in RCs increased parallel to the degree of p53 expression. Bax and Bcl-2 were detected in some basal epithelial cells in RCs and RRCs as well as in gingiva. The ssDNA reactivity significantly increased parallel to Bax expression and slightly decreased parallel to Bcl-2 expression in lining epithelium of RCs. Caspase-3 was detected in superficial epithelial cells of both gingiva and lining epithelium of RCs and RRCs, and the distribution of these cells was compatible with the expression of ssDNA. Expression of Ki-67 and ssDNA in caspase-3-positive fields was significantly higher than that in caspase-3-negative fields in RCs. There was very limited expression of Fas and Fas-L in lining epithelium of RCs and RRCs as well as in gingiva.

CONCLUSIONS: These data suggest that apoptosis-related factors are involved in the pathophysiologic activity of periapical inflammatory lesions. Such factors may be affected by the structure of lining epithelium and the degree of inflammatory change.

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Keywords: apoptosis; immunohistochemistry; lining epithelium; radicular cyst

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Introduction

Radicular cysts (RCs) are the most common jaw cysts occurring at tooth apices and develop after pulpal infection and necrosis because of dental caries (1, 2). Malassez epithelial rests may proliferate and finally form the lining epithelium of RCs (2). Residual radicular cysts (RRCs) persist after removal of teeth affected by RCs (3). Activation and proliferation of Malassez epithelial rests and lining epithelium of RCs or RRCs are related to inflammatory processes (4).

Apoptosis is physiologic cell death, involving characteristic cell shrinkage with preservation of organelles, nuclear condensation, and DNA fragmentation, accompanied by characteristic single-stranded DNA (ssDNA) production (5–9). Apoptotic reactions play various roles in the organization of normal tissues and pathogenesis and are modulated by several proteins. *p53* gene is classified as a tumor-suppressor gene, and its product plays a role in triggering apoptosis (10, 11). Mutation of this gene often results in production of mutant p53 protein, associated with cell proliferation in various cancers (10, 11). Bax and Bcl-2 are Bcl-2 family proteins distributed in the cytoplasm, and their interaction has an important part in regulating apoptosis (12–16). Bax activates apoptotic pathways, releasing cytochrome c

from the mitochondria, while Bcl-2 inhibits apoptosis, depressing the membrane permeability of the mitochondria (16). Fas (CD95) and Fas ligand (Fas-L) belong to the tumor necrosis factor (TNF) receptor family (17–19). Fas is a cell-surface glycoprotein that transmits apoptotic signals from the cell surface to the cytoplasm, while Fas-L functions as an essential effector molecule triggering apoptotic reactions (17–19). Apoptosis depends on activation of downstream executioner caspases, and activated caspase-3 cleaves various cellular proteins to cause morphologic changes of cells (19–21).

Our previous study confirmed inflammatory cells in periapical inflammatory lesions, suggesting that CD1a-positive Langerhans cells might be associated with inflammatory reaction and epithelial proliferation (22). Some studies provide evidence that apoptotic mechanisms are implicated in the inflammatory processes of marginal periodontal lesions (23, 24). However, the role of apoptotic cell death in periapical inflammatory lesions remains unclear. To clarify possible roles of apoptosis in periapical inflammatory lesions, we immunohistochemically studied apoptosis and apoptosis-related factors, including ssDNA, p53, Bax, Bcl-2, caspase-3, Fas, and Fas-L, in RCs and RRCs. Ki-67 antigen immunoreactivity, a cell proliferation marker (25), was also examined and contrasted with apoptotic cell death and apoptosis-related factors.

Materials and methods

Tissue preparation

A total of 19 RCs (obtained from 13 men and six women aged 43.8 ± 13.0 years) and five RRCs (obtained from three men and two women aged 50.2 ± 22.3 years) were collected from banked tissue blocks prepared by the Division of Oral Pathology, Department of Oral Medicine and Bioregulation, Tohoku University Graduate School of Dentistry. All specimens were fixed in 10% buffered formalin for several days and were embedded in paraffin wax. Serial sections 3 μm thick were sliced from the tissue blocks and were processed for subsequent histologic and immunohistochemical studies. At least one section of each specimen was stained with hematoxylin and eosin and examined morphologically. The other sections were processed for subsequent immunohistochemical study. As control, specimens of normal gingivae excised from seven cadavers undergoing autopsy at Tohoku University Medical Hospital were similarly prepared and examined. We have obtained the normal gingivae within about an hour from death to obviate the influence of necrotic changes.

Immunohistochemistry

Tissue sections were deparaffinized and immersed in methanol with 3% hydrogen peroxide to eliminate endogenous peroxidase activity. Several sections for Ki-67, p53, Bax, Bcl-2, and caspase-3 were boiled for antigen retrieval by autoclave in 0.01 M citrate buffer (pH 6.0) for 10 min (120°C, 2 atm). After treatment with normal rabbit or goat serum for 20 min to block non-specific binding, the sections was incubated with primary antibodies at 4°C overnight. The primary antihuman antibodies used are shown in Table 1. The standard streptavidin-biotin-peroxidase complex method was performed to bind the primary antibodies with the use of Histofine SAB-PO Kits (Nichirei, Tokyo, Japan). Briefly, biotin-labeled anti-immunogloblin antibodies and peroxidase-conjugated streptavidin were reacted, and then reaction products were visualized by immersing the sections for 3–5 min in 0.03% 3,3-diaminobenzidine (DAB) solution containing 2 mM hydrogen peroxide. Nuclei were lightly counterstained with 1% methyl green.

Evaluation of staining behavior and statistical analysis

The proportions of proliferative and apoptotic cells in the lining and gingival epithelium were expressed by labeling indices for Ki-67 antigen and ssDNA immunostaining (Ki-67-LI and ssDNA-LI), defined as the mean ratio of positive cells among 1000 epithelial cells in 10 randomly selected fields. Immunohistochemical reactivity for p53, Bax, Bcl-2, caspase-3, Fas, and Fas-L was classified into three categories according to the percentage of positively stained nuclei (p53 and caspase-3) or cytoplasm (Bax, Bcl-2, Fas, and Fas-L) of the lining and gingival epithelium in the entire sections as follows: (−), no staining (5% or less of epithelial cells positive); (+), slight to moderate staining (more than 5% to <25% of epithelial cells positive); (2+), intense staining (25% or more of epithelial cells positive). Four fields were selected in each lining epithelium of 19 RCs, and a total of 76 fields were examined for analysis of correlations between histopathologic and immunohistochemical findings, and between cellular activities and apoptosis-related factors of RCs. Differences in mean Ki-67-LI and ssDNA-LI were analyzed by the Mann–Whitney *U*-test, while the statistical significance of differences in p53, Bax, Bcl-2, caspase-3, Fas, and Fas-L reactivity

Table 1 Primary antibodies used for immunohistochemical studies

Antigen	*Clonality*	*Dilution*	*Pre-treatment*	*Source*
Ki-67 (MIB-1)	Monoclonal	Pre-diluted	Autoclave	Immunotech, Marseille, France
Single-stranded DNA (ssDNA)	Polyclonal	1:100	None	Dako, Glostrup, Denmark
p53 protein (DO-7)	Monoclonal	1:50	Autoclave	Dako
Bax protein (N20)	Polyclonal	1:1000	Autoclave	Santa Cruz Biotechnology, Santa Cruz, CA, USA
Bcl-2 protein (Clone 124)	Monoclonal	1:40	Autoclave	Dako
Caspase-3 (CPP32)	Polyclonal	1:200	Autoclave	Dako
Fas (CD95)	Polyclonal	1:200	None	Wako, Osaka, Japan
Fas ligand (Fas-L)	Polyclonal	1:200	None	Wako

were analyzed by the chi-square test. *P*-values of <0.05 were considered to indicate statistical significance.

Results

All periapical lesions showed various degrees of inflammatory cell infiltration (Fig. 1b–d, f–h). Gingival tissue (Fig. 1a,e), used as control, showed minimal inflammatory cells, and RRCs showed less inflammatory cell infiltration than RCs. Periapical lesions had diffuse inflammatory cell infiltration in the inner granulation tissue and perivascular infiltration in the outer fibrous tissue. Several inflammatory cells infiltrated within the epithelial components in these lesions. Lining epithelium of RCs and RRCs revealed various degrees of thickness or elongated rete processes.

Immunohistochemical findings in the epithelium of gingiva, RCs, and RRCs

In gingiva, RCs, and RRCs, Ki-67 was expressed in the nuclei of basal and parabasal cells of the epithelium (Fig. 1a–d), whereas ssDNA was expressed in the nuclei of suprabasal and superficial cells of the epithelium (Fig. 1e–h). Both the mean Ki-67-LI and ssDNA-LI in RCs and RRCs were slightly higher than those in gingiva. Immunohistochemical reactivity for p53 was detected in the nuclei of basal and parabasal cells of the epithelium in gingiva, RCs, and RRCs (Fig. 2a). Expression of Bax and Bcl-2 was detected in the cytoplasm of basal and parabasal cells (Fig. 2b,c), and caspase-3 was recognized in the nuclei of superficial cells (Fig. 2d). Fas and Fas-L were not detected in gingiva, and expression of Fas and Fas-L was limited in lining epithelium of RCs and RRCs. Expressions of Ki-67, ssDNA, p53, Bax, Bcl-2, caspase-3, Fas, and Fas-L were detected in some subepithelial inflammatory cells in RCs and RRCs. Expression of p53 in RCs was significantly greater than that in gingiva ($P < 0.05$). Expression of Bax, Bcl-2, and caspase-3 was slightly greater in RCs and RRCs than in gingiva. Fas and Fas-L showed no significant differences among gingiva, RCs, and RRCs.

Correlation between histopathologic and immunohistochemical findings in RCs

Mean Ki-67-LI in lining epithelium with rete processes, with intense subepithelial inflammation, or with 10 cell layers or more were significantly higher than those in epithelium without rete processes, with no or less subepithelial inflammation, or with <10 cell layers ($P < 0.001$, 0.01, and 0.001, respectively). Mean ssDNA-LI in lining epithelium with intense subepithelial inflammation, or with 10 cell layers or more were significantly higher than those in epithelium with no or less subepithelial inflammation, or with <10 cell layers ($P < 0.05$, and 0.001, respectively). Lining epithelium with 10 cell layers or more showed significantly higher expression of p53, Bax, and caspase-3 than did lining epithelium with <10 cell layers (p53: $P < 0.01$; Bax: $P < 0.05$; caspase-3: $P < 0.001$). Expression of Bax in lining epithelium with intense chronic inflammatory cell infiltration was significantly higher than that with no or less chronic inflammatory cell infiltration ($P < 0.01$), whereas expression of Bcl-2 in lining epithelium with intense inflammation was significantly lower than that with no or less inflammation ($P < 0.001$). Expression of caspase-3 in lining epithelium with rete processes was significantly higher than that without rete processes ($P < 0.05$), whereas expression of Bcl-2 in lining epithelium with rete processes was significantly lower than that without rete processes ($P < 0.001$). No apparent correlation was recognized between Fas or Fas-L expression and the morphologic characteristics of lining epithelium in RCs.

Correlation between cellular activities and apoptosis-related factors in RCs

The Ki-67-LI and ssDNA-LI in p53 intensely positive cases were significantly higher than those in slightly to moderately positive cases and those in negative cases (Ki-67-LI: $P < 0.05$ and $P < 0.001$; ssDNA-LI: $P < 0.05$ and $P < 0.05$, respectively). p53 slightly to moderately positive cases had a significantly higher Ki-67-LI than negative cases ($P < 0.05$). There was no

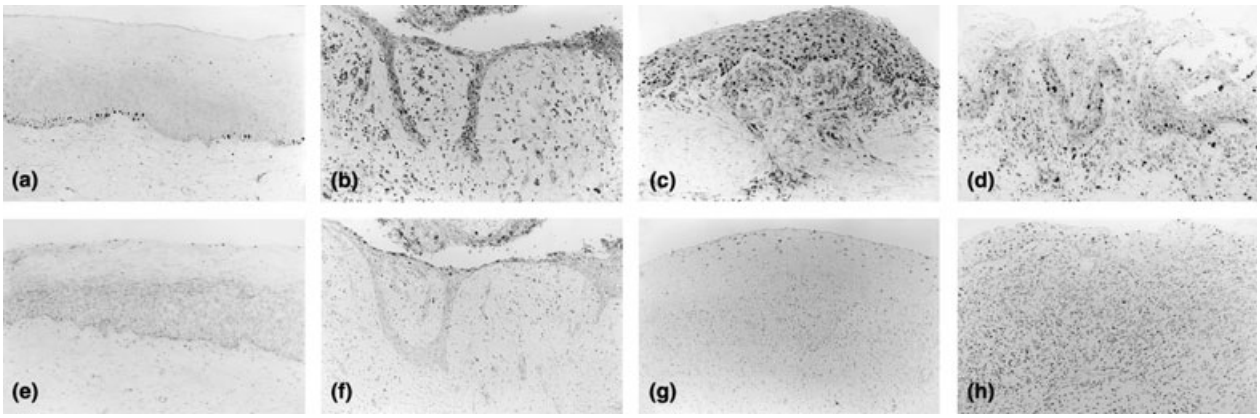

Figure 1 Immunohistochemical reactivity for Ki-67 and single-stranded DNA (ssDNA) in gingiva, lining epithelium of radicular cysts (RCs) and residual radicular cysts (RRCs). Expression of Ki-67 in gingiva (a), lining epithelium of RCs with proliferating rete processes (b) and without rete processes (c), and lining epithelium of RRCs (d) was observed in the nuclei of basal and parabasal cells. Expression of ssDNA in gingiva (e), lining epithelium of RCs with proliferating rete processes (f) and without rete processes (g), and lining epithelium of RRCs (h) was observed in the nuclei of suprabasal and superficial cells. (a,e: ×40; b–d, f–h: ×55)

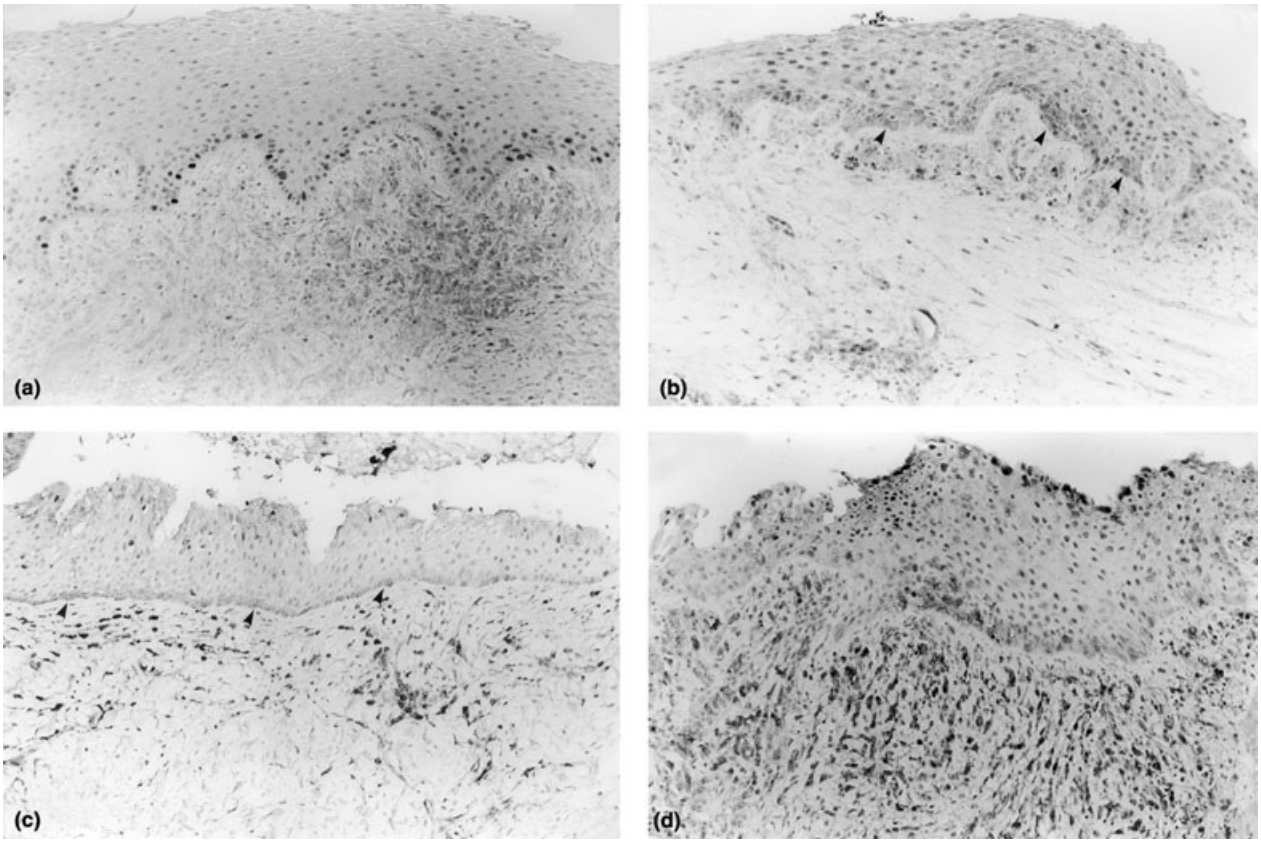

Figure 2 Immunohistochemical reactivity for apoptosis-related factors in lining epithelium of radicular cysts (RCs). Expression of p53 (a) was detected in the nuclei of basal and parabasal cells. Expression of Bax (b) and Bcl-2 (c) was detected in the cytoplasm of basal and parabasal cells (arrowheads). Infiltrating inflammatory cells were also positive for Bax (b) and Bcl-2 (c). Expression of caspase-3 (d) was detected in the nuclei of superficial cells of lining epithelium and subepithelial inflammatory cells. (×110)

significant correlation between Ki-67-LI and Bax expression, whereas ssDNA-LI in Bax-positive cases was significantly higher than that in negative cases ($P < 0.01$). Ki-67-LI in Bcl-2-positive cases was significantly lower than that in negative cases ($P < 0.001$); there was no significant correlation between ssDNA-LI and Bcl-2 expression. Ki-67-LI and ssDNA-LI in caspase-3 intensely positive cases were significantly higher than those in negative cases (Ki-67-LI: $P < 0.01$; ssDNA-LI: $P < 0.05$), and Ki-67-LI in caspase-3 intensely positive cases was significantly higher than that in slightly to moderately positive cases ($P < 0.01$). There was no significant correlation of Ki-67-LI or ssDNA-LI with expression of Fas or Fas-L.

Discussion

Since the growth rate or activity of inflammatory lesions depends on the balance between cell proliferation and death (26), we examined immunoreactivity for Ki-67 antigen as a proliferation marker in addition to apoptotic reactions, including ssDNA formation. In our previous study, Ki-67 antigen was expressed in nuclei of the basal cells in lining epithelium of RCs and RRCs (22). The antibody against ssDNA is a sensitive marker for the detection of only apoptotic cells, especially those in critical early apoptosis (9), then we have considered ssDNA-positive epithelial cells as apoptotic cells in this study. In odontogenic keratocysts, expression of ssDNA was detected in the nuclei of superficial cells of lining epithelium (27). In the present study, epithelial ssDNA expression was detected also in the nuclei of superficial cells. Mean Ki-67-LI and ssDNA-LI in RCs and RRCs were slightly higher than those in gingiva, and both indices in RCs were slightly higher than those in RRCs. Both Ki-67-LI and ssDNA-LI in RCs with intense inflammatory cell infiltration were significantly higher than those in RCs with less inflammatory reactions. These results suggest that proliferative activity and apoptosis in lining epithelium of RCs have different characteristics from those in normal stratified squamous epithelium and that cellular kinetics or turnover of cyst lining epithelium might be related to the grade of inflammatory changes (22). In the present study, there seemed to be a higher proliferation than apoptosis in lining epithelium of RCs (Tables 2–4). We consider that it is hard to compare Ki-67-LI and ssDNA-LI, because Ki-67-LI is higher than ssDNA-LI in control gingival epithelium in Table 2, or because our results show much smaller difference between Ki-67-LI and ssDNA-LI than other lesions such as tumors. The difference between Ki-67-LI and ssDNA-LI might be partly due to the different immunohistochemical procedure using monoclonal anti-Ki-67 antibody and polyclonal anti-

Table 2 Immunohistochemical findings of cellular activities and apoptosis-related factors in gingiva and lining epithelium of radicular cysts (RCs) and residual radicular cysts (RRCs)

	Labeling index		*p53*			*Bax*		*Bcl-2*		*Caspase-3*			*Fas*		*Fas-L*	
	Ki-67-LI	*ssDNA-LI*	−	+	2+	−	+	−	+	−	+	2+	−	+	−	+
Gingiva ($n = 7$)	1.14 ± 0.45	0.24 ± 0.14	6 (86)	1 (14)	0 (0)] *	4 (57)	3 (43)	7 (100)	0 (0)	4 (57)	3 (43)	0 (0)	7 (100)	0 (0)	7 (100)	0 (0)
RC ($n = 19$)	1.59 ± 0.82	0.36 ± 0.25	4 (22)	13 (67)	2 (11)] *	10 (55)	9 (45)	17 (89)	2 (11)	10 (56)	5 (32)	4 (22)	17 (89)	2 (11)	16 (84)	3 (16)
RRC ($n = 5$)	1.34 ± 0.34	0.28 ± 0.19	2 (40)	2 (40)	1 (20)	2 (40)	3 (60)	4 (80)	1 (20)	2 (40)	3 (60)	0 (0)	4 (80)	1 (20)	5 (100)	0 (0)

Values in parentheses expressed as percentage.
Immunohistochemical reactivity: (−) no staining, (+) slight to moderate staining, (2+) intense staining; ssDNA, single-stranded DNA.
Statistical significance: $*P < 0.05$.

Table 3 Immunohistochemical findings of cellular activities and apoptosis-related factors in lining epithelium of radicular cysts

	Number of fields	*Labeling index*		*p53*			*Bax*		*Bcl-2*		*Caspase-3*			*Fas*		*Fas-L*	
		Ki-67-LI	*ssDNA-LI*	−	+	2+	−	+	−	+	−	+	2+	−	+	−	+
Morphology of lining epithelium																	
With rete processes	50	3.74 ± 0.84] ***	0.44 ± 0.45	11	29	10	23	27	48	2] ***	24	12	14] *	44	6	47	3
				(22)	(58)	(20)	(46)	(54)	(96)	(4)	(48)	(24)	(28)	(88)	(12)	(94)	(6)
Without rete processes	26	2.67 ± 1.23] ***	0.52 ± 0.40	4	20	2	9	17	17	9] ***	8	14	4	23	3	26	0
				(16)	(76)	(8)	(35)	(65)	(65)	(35)	(33)	(51)	(16)	(89)	(11)	(100)	(0)
Chronic inflammatory cells																	
Intense infiltration	55	3.67 ± 0.98] **	0.54 ± 0.46] *	9	35	11	18	37] **	54	1] ***	25	17	13	47	8	52	3
				(16)	(64)	(20)	(32)	(68)	(98)	(2)	(46)	(31)	(23)	(86)	(14)	(95)	(5)
No or less infiltration	21	2.62 ± 1.06] **	0.28 ± 0.27] *	6	14	1	14	7] **	11	10] ***	7	9	5	20	1	21	0
				(28)	(67)	(5)	(67)	(33)	(52)	(48)	(34)	(52)	(24)	(95)	(5)	(100)	(0)
Number of layers of lining epithelium																	
Less than 10 cell layers	44	2.98 ± 0.86] ***	0.30 ± 0.39] ***	13	29	2] **	23	21] *	35	9	28	11	5] ***	39	5	43	1
				(30)	(65)	(5)	(52)	(48)	(79)	(21)	(63)	(25)	(12)	(89)	(11)	(98)	(2)
10 cell layers or more	32	3.94 ± 0.92] ***	0.69 ± 0.34] ***	2	20	10] **	9	23] *	30	2	4	15	13] ***	28	4	30	2
				(6)	(62)	(32)	(28)	(72)	(94)	(6)	(12)	(48)	(40)	(88)	(12)	(94)	(6)

Values in parentheses expressed as percentage.
Immunohistochemical reactivity: (−) no staining, (+) slight to moderate staining, (2+) intense staining; ssDNA, single-stranded DNA.
Statistical significance: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Table 4 Immunohistochemical findings of cellular activities and apoptosis-related factors in lining epithelium of radicular cysts

	Number of fields	*Labeling index*	
		Ki-67-LI	*ssDNA-LI*
p53			
−	15	2.60 ± 0.93	0.31 ± 0.41
+	49	3.40 ± 1.01	0.45 ± 0.43
2+	12	4.29 ± 0.99	0.73 ± 0.37
Bax			
−	32	3.20 ± 1.05	0.29 ± 0.38
+	44	3.51 ± 1.13	0.60 ± 0.43
Bcl-2			
−	65	3.58 ± 1.00	0.50 ± 0.45
+	11	2.18 ± 0.90	0.27 ± 0.31
Caspase-3			
−	32	3.20 ± 1.89	0.36 ± 0.48
+	26	3.10 ± 1.12	0.52 ± 0.41
2+	18	4.11 ± 1.14	0.59 ± 0.34
Fas			
−	67	3.31 ± 1.07	0.46 ± 0.43
+	9	3.89 ± 1.27	0.49 ± 0.48
Fas-L			
−	73	3.36 ± 1.11	0.46 ± 0.43
+	3	3.83 ± 1.04	0.60 ± 0.60

Immunohistochemical reactivity: (−) no staining, (+) slight to moderate staining, (2+) intense staining; ssDNA, single stranded DNA. Statistical significance: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

ssDNA antiserum. Apoptosis-related factors used in this study have been demonstrated in subepithelial inflammatory cells of gingiva with periodontitis or buccal mucosa with oral lichen planus (24, 28). Their staining patterns suggest that apoptosis is involved in the pathogenesis of these diseases. RCs with marked rete process formation showed higher proliferative activity than RCs with flat lining epithelium; however, apoptotic reactions did not differ significantly between these RCs. Both proliferative activity and apoptotic cell death in RCs with thick lining epithelium were greater than those in RCs with thin lining epithelium. We have not studied the morphologic features such as the formation of apoptotic bodies by using electron microscope, but we have considered ssDNA-positive epithelial cells as apoptotic cells. A further series of the study to distinguish nuclear changes associated with epithelial differentiation from classical apoptosis would be necessary in future. The expressions of some transcription factors such as p63, p107, or p130 are believed to have an important role in epithelial stratification and terminal differentiation (29).

The *p53* gene product plays an important role in the response to DNA damage, regulating G1 phase arrest and apoptosis (11). Normal oral mucosa and benign lesions are usually negative to slightly positive for p53 protein (30–33); however, in some cases, p53 was expressed because of an increase in non-mutant forms of p53 (34). We used a monoclonal antibody reactive with wild type and mutant type p53 proteins, and immunohistochemical reactivity for p53 was noted sporadically in the lining epithelium of RCs and RRCs but not in normal gingival epithelium. We also found that Ki-67 and ssDNA reactivity of RCs increased according to the degree of p53 expression. Immunohistochemical detection of p53 protein indicates either increased production or decreased breakdown of the protein and is related to cell proliferation (30). Our results suggest that p53 expression might reflect both cellular proliferative potential and induction of apoptosis in RCs and RRCs.

Immunoreactivity for Bcl-2 family proteins has been detected in odontogenic epithelium under various conditions, suggesting that these proteins play a role in regulating cellular kinetics of odontogenic epithelium (35, 36). In odontogenic keratocysts, epithelial Bcl-2 expression is sometimes decreased when the epithelium is infiltrated by inflammatory cells (37). In the present study, Bax and Bcl-2 were detected in some basal epithelial cells of RCs and RRCs as well as in gingival stratified squamous epithelium. RCs with intense inflammatory changes showed higher Bax expression and lower Bcl-2 expression than RCs with less inflammatory changes. Mean ssDNA-LI was higher in Bax-positive fields and in Bcl-2-negative fields in RCs. These results suggest that these Bcl-2 family members regulate apoptosis in some balance (31, 38) that contributes to proliferation and cell death of lining epithelium of inflammatory periapical lesions. Our findings also suggest that Bcl-2 family members are affected by inflammatory processes in these lesions.

Apoptosis depends on caspase activity, and expression of caspase-3 has been considered essential for apoptosis activation (21, 24). Caspase-3-positive cells have been demonstrated in lining epithelium of odontogenic keratocysts or neoplastic cells of ameloblastomas (27, 39). In the present study, caspase-3 was detected in superficial cells in both gingiva and lining epithelium of RCs and RRCs, and the distribution of these cells was compatible with the expression of ssDNA. Mean Ki-67-LI and ssDNA-LI in caspase-3-positive fields were higher than those in negative fields in RCs, suggesting that increased caspase-3 expression reflects up-regulation of not only apoptosis but also of proliferation of cyst lining epithelium (27).

The Fas/Fas-L system is functional in normal tissues, including the immune system, and its abnormality causes various pathologic conditions, such as immunologic disorders and neoplasms (39). Recent studies have reported Fas and Fas-L expression in odontogenic lesions (27, 39). In the present study, expression of Fas and Fas-L was low in lining epithelium of RCs and RRCs, and no correlation among these factors and various variables were detected. These features suggest that Fas, Fas-L, or both most likely do not play a major role in cell proliferation or apoptotic cell death in lining epithelium of RCs and RRCs.

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